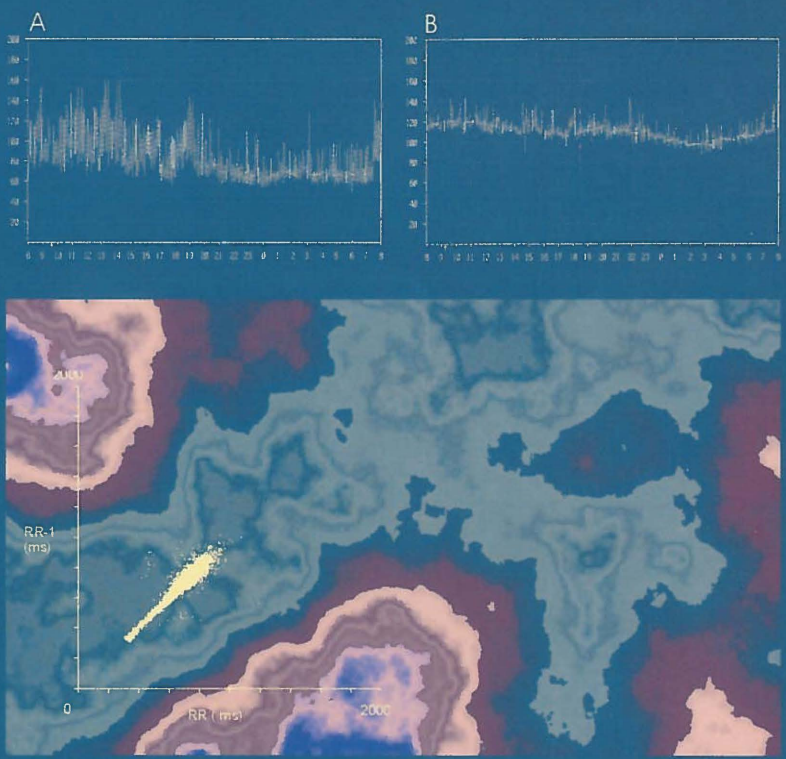
Heart rate variability in cardiology. Methodological and clinical aspects.

Haaksma, Jacob

Heart Rate Variability in cardiology

Methodological and clinical aspects



J. Haaksma

Heart Rate Variability in Cardiology
Methodological and clinical aspects

J. Haaksma

Stellingen behorend bij het proefschrift

Heart Rate Variability in Cardiology

J. Haaksma

1. Een betrouwbare analyse van HRV begint bij de opname van het electrocardiogram.
2. HRV kan zonder consistente en nauwkeurige detectie van QRS-complexen niet betrouwbaar worden vastgesteld.
3. Het gebruik van procentuele exclusieregels is kwalitatief niet verantwoord, *manual editing* is noodzakelijk.
4. *Discrete* Fourier transformatie is zowel theoretisch als praktisch te prefereren boven *fast* Fourier transformatie voor de analyse van HRV.
5. Het opleggen van een vaste ademhalingsfrequentie biedt geen voordelen boven spontaan ademen bij het analyseren van de HRV.
6. De bepaling van HRV is pas representatief voor een dag indien de registratieduur ten minste 22 uur bedraagt.
7. De analyse van HRV kan het vermoeden op een vagale genese van boezemfibrilleren bevestigen.
8. Tijdens boezemfibrilleren is HRV gerelateerd aan de vagale tonus.
9. HRV-analyse suggereert dat vrouwen snelle mannen zijn.
10. HRV is als risico-indicator na het myocardinfarct een goede aanvulling op klassieke variabelen zoals de ejectiefractie van de linker kamer en de *New York Heart Association* classificatie (Odemuyiwa Am J Cardiol 1991).
11. Technische artikelen worden door medici veelal ondergewaardeerd terwijl technici te weinig oog hebben voor medisch-praktische oplossingen.
12. Het ontbreken van een kwaliteitsnorm voor Holters is een slechte zaak.
13. HRV is minder belangrijk dan luisteren naar een patiënt.
14. Luisteren naar een patiënt dient niet alleen door de stethoscoop te gebeuren.
15. Het is inconsequent om wel menugestuurde statistiekprogramma's te accepteren maar niet menugestuurde medische diagnoseprogramma's.
16. Dat bij HRV geldt dat chaos goed is en orde slecht, betekent niet dat Feyenoord nog eens landskampioen mag worden.

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

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Methodological and clinical aspects

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”We are prone to judge success by the index of our salaries
or the size of our automobiles,
rather than by the quality of our service
and relationship to humanity.”

Martin Luther King

Voor mijn grootvader en mijn moeder

TABLE OF CONTENTS

1
INTRODUCTION AND AIM OF THE THESIS

Centuries ago, an interaction between the heart and the nervous system was postulated. After the description of the circulatory system by Sir William Harvey, Dr. Thomas Willis was the first to describe the basics of "neuro-cardiology". In 1664 he published "Cerebri anatome", the basic text on the anatomy of the central nervous system. In this document he suggested that nerves are conductors rather than hollow tubes and that animal spirits move about the body more mysteriously than water in plumbing. One of his observations was that the supposedly regular rhythm of the heart was not precisely regular. Nowadays, analysis of Heart Rate Variability (HRV) is used to study these irregularities in heart rate [31, 34, 36, 228, 236]. In recent years this technique has gained increasing popularity because it is a non invasive tool to study the interaction between the autonomic nervous system and the heart. However, the application of HRV analysis and its interpretation are rather complex. The implementation of HRV analysis is even more complicated because different techniques are used. Moreover, HRV analysis is a mixture of both medical and mathematical concepts, which implies that knowledge of both disciplines is required to appreciate all the possibilities of HRV analysis.

There are many factors that may affect the outcome of HRV analysis. Apart from the physiological mechanisms that are the aim of HRV analysis, many technical aspects may interfere. In 1996 a Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology published a special report on HRV called "Standards of Measurement, Physiological Interpretation and Clinical Use"[1, 2]. This report is referred to as "*the guidelines*" from here on in this thesis. These guidelines seek to define theoretical, technical and practical limitations for the use of HRV. Although this is a comprehensive piece of work, many questions remain unanswered. The application of several preparatory steps and analysis methods as well as HRV variables, have made it difficult - if not impossible - to compare studies. Therefore, it is necessary to use reliable analysis methods, limit their number and to provide insight into the consequences of using certain analysis methods. In other words, further standardisation is needed.

No clear directives are present in the guidelines with respect to minimally required recording duration, maximal percentage of ectopic beats that can be accepted for analysis and other practical questions relating to effects of breathing and QRS width. Furthermore, the application of HRV analysis is discussed only with respect to sinus rhythm. On the one hand this strategy relates to the fact that the autonomic nervous system mainly affects the rhythm through the sinus node and to a lesser extent the atria, atrioventricular node and ventricles. On the other hand, irregularities of the rhythm caused by ectopic beats or atrial fibrillation may be largely independent of autonomic tone. As a consequence, ectopic beats are usually excluded prior to calculation of HRV variables[83]. Similarly, HRV analysis using all RR-intervals is considered

unreliable[2]. The remainder of a RR-interval series from which intervals adjacent to ectopic beats are excluded, are called normal-to-normal (NN) intervals. Still, analysis of the RR-interval variation in dysrythmias such as atrial fibrillation may yield clinically significant results. Specifically it may provide insight into the evoking as well as preserving mechanism of this rhythm disorder.

The aim of this thesis is, therefore, to assess existing methods of HRV analysis and discuss the consequences of the findings for clinical use, to provide directives for practical use of HRV analysis in the field of cardiology. Also, practical details discussed in the guidelines are more critically considered. The optimal analysis method found in this way will be used to assess the applicability of HRV analysis in the setting of atrial fibrillation.

Chapter 2 is a summary of the thesis in which the background of HRV as well as methodological aspects that may influence the outcome of HRV analysis are described. Chapter 3 gives an overview of the basis for appropriate HRV analysis, which is ECG recording and analysis. Further methodological aspects of HRV analysis are discussed in more detail in chapters 4 to 8. The importance of the above is illustrated in chapter 9, detailing specific aspects of HRV analysis in patients with atrial fibrillation. The nomenclature of HRV variables used in this thesis is explained in Table 1, the other abbreviations are listed in Table 2

variable	units	description
NN		Normal to normal interval: RR-intervals started and ended by a beat originating from the sinus node
AVGNN	ms	Average of all NN-intervals
SDNN	ms	Standard deviation of all NN-intervals
SDANN	ms	Standard deviation of the average NN-intervals computed over 5-minute segments
SDNNindex	ms	Average of all standard deviations of NN-intervals computed over 5-minute segments
rMSSD	ms	root mean square of successive differences
TP	ms^2	Total Power (< 0.40 Hz)
VLF	ms^2	Very low frequency power (0.033 - 0.04 Hz)
LF	ms^2	Low frequency Power (0.04 - 0.15 Hz)
HF	ms^2	High frequency Power (0.15 - 0.40 Hz)
lnVLF		natural logarithm of VLF
lnLF		natural logarithm of LF
lnHF		natural logarithm of HF
LFnu	%	normalized LF power (LF/TP)*100
HFnu	%	normalized HF power (HF/TP) * 100
LFHF		LF/HF
ccvVLF	%	$(\sqrt{sqrt(VLF)}/AVGNN)*100\%$
ccvLF	%	$(\sqrt{sqrt(LF)}/AVGNN)*100\%$
ccvHF	%	$(\sqrt{sqrt(LF)}/AVGNN)*100\%$

Table 1. HRV variables, names and definitions.

Abbreviation	Explanation
AD conversion	Analog to digital conversion
CCV	Component coefficient of variation
DFT	Discrete Fourier Transformation
ECG	Electrocardiogram
FFT	Fast Fourier Transformation
HRV	Heart Rate Variability
Hz	Hertz
NSI	Non sinus intervals
PVC	Premature Ventricular Contraction

Table 2. List of abbreviations used in this thesis.

2
HEART RATE VARIABILITY ANALYSIS

2.1 INNERVATION OF THE HEART

Histological studies have shown that the sinus node is the most richly innervated part of the heart [190]. The sinus node is influenced by the two divisions of the autonomic nervous system. While the sympathetic limb accelerates heart rate, the parasympathetic or vagal limb causes deceleration. Usually, vagal activity is predominant in healthy individuals at rest[6, 109]. The vagal innervation originates from the medulla oblongata in cells that are located in the dorsal motor nucleus or the nucleus ambiguus. The innervation of the left and right vagus nerves is not equally distributed over the heart as shown in animal[197-199] as well as human[57, 107] studies. While the sinus node is mainly innervated by the right vagus nerve, the atrioventricular node, and hence atrioventricular conduction, is mainly influenced by the left vagus nerve. The cardiac sympathetic fibers originate in the intermediolateral columns of the upper thoracic and lower cervical segments of the spinal cord. Within the sympathetic division, the distribution between left and right sympathetic fibers is also different. While the right-sided sympathetic fibers mainly influence the sinus node, the left-sided fibers predominantly affect myocardial contractility[140].

2.2 PHYSIOLOGICAL BACKGROUND OF VARIATIONS IN SINUS RHYTHM

In 1934, Rosenblueth and Simeone described a model[205] which states that the actual heart rate is considered the result of a basic or intrinsic heart rate (HR_0) modulated by the autonomic nervous system. The intrinsic heart rate is multiplied by a decreasing factor (n), representing vagal influence and an increasing factor (m), representing sympathetic influence. Therefore, the sympathovagal balance can be defined as the product of the sympathetic and vagal influence. Accordingly, the actual heart rate can be expressed as $HR = m \cdot n \cdot HR_0$. In this expression, m is greater than or equal to 1 and n is less than or equal to 1. Implicit to this model is, that the precise values of m and n cannot be computed without the use of autonomic blockade. However, assuming that the intrinsic heart rate is a rather constant value within a subject, this also means that the actual heart rate is itself a marker of the sympathovagal balance. It is important to recognize that changes in HRV represent changes in sympathovagal balance. Therefore, changes in HRV reflect fluctuations of (para)sympathetic tone, not changes in sympathetic tone or vagal tone itself[2, 146]. Based on an identical intrinsic heart rate, the same actual heart rate can be obtained using different levels of sympathetic and vagal tone (Table 3).

HR (BPM)	m	n	HR_0 (BPM)
60	1.5	1	90
60	2	0.75	90
60	1.8	.8334	90

Table 3.
Relation between actual and intrinsic heart rate. According to the Rosenblueth Simeone model ($HR = m \cdot n \cdot HR_0$) different levels of vagal (m) and sympathetic (n) tone may lead to the same actual heart rate, based on the same intrinsic heart rate.

The mechanisms that cause vagal and sympathetic activity to fluctuate are complex. There are several mechanisms governing heart rate, e.g. those active through baroreceptors, chemoreceptors and atrial receptors. Stimulation of these receptors may have varying effects, depending on factors such as average blood pressure[63] or location of the receptors[229]. Moreover the magnitude of effect, as well as the intrinsic mechanism by which these receptors have an effect, differs. Well known clinically is the so-called respiratory sinus arrhythmia. The different mechanisms leading to respiratory sinus arrhythmia are shown in Figure 1.

These mechanisms can be divided into 2 groups:

I) Neurally evoked mechanisms:

- A direct effect of the respiratory control centre on the cardiovascular control centre leading to vagal inhibition during inspiration[92, 128].
- Stretch receptors located in the lungs influence the cardiovascular control centre, also leading to inhibition of vagal output to the heart during inspiration[70, 71].
- Right atrial stretch increases heart rate, however not after vagotomy. This is called the Bainbridge reflex[12, 13].
- A lowering of the intrathoracic pressure as a consequence of inspiration results in a larger pressure difference over the aortic wall. This causes a baroreflex response resulting in a lowering of the vagal output to the heart.

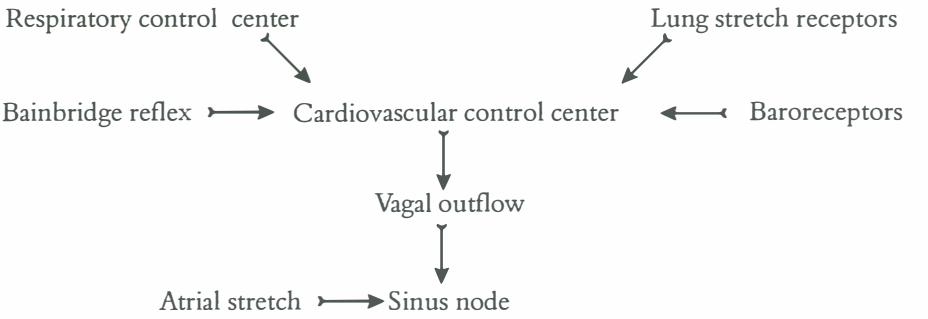


Figure 1. Mechanisms leading to respiratory sinus arrhythmia. Local atrial stretch, reflex feedback and direct brain mechanisms cause sinus arrhythmia.

II) A direct or local mechanism:
- Heart rate lowers during inspiration in a denervated heart, possibly due to mechanical coupling as a result of varying intrathoracic pressure and a resulting change in venous return[17].

2.3 PRACTICAL APPLICATION OF HRV ANALYSIS

Ideally, the status of the autonomic nervous system is assessed by simultaneous recording of additional parameters besides heart rate such as blood pressure or respiration[41, 176]. Since several interactions exist, a combined registration of various parameters may provide better insight into the autonomic regulation of the cardiovascular system. In this respect, the interaction between blood pressure and heart rate has been most extensively evaluated. A model for blood pressure control was developed by Wesseling, Settels and co-authors[256, 257] and was later extended by van Roon[204]. However, the necessary parameters cannot easily be obtained in the clinical setting. Therefore, while it seems attractive to use a more complete model, it should be kept in mind that the "drawbacks" of a simple model can also be an advantage. The easiness of use makes such a model widely applicable[2].

Although RR-interval sequences are analysed, for practical purposes actually PP intervals are of interest. Any difference between PP and RR-interval series is due to the influence of the atrioventricular node. The atrioventricular conduction adapts to heart rate[136] in a similar fashion but to a lesser extent as the sinus node[185, 254, 255], however the amount is generally considered to be negligible. In 1973, Sayers[212] stated that it is possible to differentiate between the different physiological factors that induce variability in heart rate by means of frequency selective analysis of cardiac inter-beat intervals. Furthermore, he postulated that fluctuations in heart rate are to a certain extent independent of heart rate itself. At the same time, Mulder & Mulder[177] showed in the same volume of Ergonomics that these variations in heart rate are influenced by mental tasks. Using frequency-domain analysis, they identified three major peaks in the heart rate spectrum, at approximately 0.1, 0.3 and 0.5 Hz. In their experiments, the 0.3 Hz peak proved to be related to respiration, while the 0.5 Hz peak was related to the stimulus frequency of repetitive mental task. In 1975 Katona and Jih[114] demonstrated a strong, linear relationship between the amount of respiratory sinus arrhythmia and the change in heart rate resulting from vagal blockade. They concluded that respiratory sinus arrhythmia may be used as a non invasive index of vagal cardiac control. In his master thesis, Mulder[175] showed the usefulness of frequency-domain analysis in the study of HRV, since different parts of the spectrum reacted differently to mental tasks. This and other studies have led to the conclusion that power spectrum analysis of heart rate is capable of distinguishing between different fluctuations in heart

rate, representing different modulation sources from the autonomic nervous system. Akselrod et al.[5] postulated a relationship between the 0.04 Hz peak and the renin-angiotensin system. In 1985, Eckberg[59] showed a strong relationship between breathing frequency and respiratory sinus arrhythmia and only a moderate relationship between tidal volume and respiratory sinus arrhythmia. Pomerantz et al[195] demonstrated that slow oscillations (< 0.12 Hz) were influenced by both vagal and sympathetic control, while faster changes were the result of vagal activity. Using short-term recordings, two frequency bands are usually recognized in the spectrum[2]:

LF: 0.04 - 0.15 Hz, representing vagal as well as sympathetic activity and
HF: 0.15 - 0.40 HZ, representing predominantly vagal activity.

Broad consensus exists about the fact that HF is predominantly the result of vagal activity, while the origin of the LF fluctuations is an ongoing matter of debate. A discussion on the origin and practical use of different frequency bands of the heart rate power spectrum is presented in chapter 2.4.3.

2.4 ANALYSIS ASPECTS OF HEART RATE VARIABILITY

2.4.1 ECG registration

Before HRV calculations can be made, a reliable ECG recording must be obtained. After analysis of the ECG, the RR-interval series of all beats has to be derived from the ECG. In the field of cardiology, HRV analysis usually is applied to RR-interval series obtained from 24-hour ambulatory ECG recordings (Holter) [40, 44, 171]. In this thesis, the source of ECG is mainly ambulatory monitoring recordings. The first step in ambulatory monitoring is the hook-up procedure. This seemingly trivial and often underestimated step may however have a major effect on the outcome of HRV analysis. In the guidelines[2], no recommendations with regard to ECG hook-up are presented. It is obvious that an inadequate registration of the ECG cannot be compensated for in subsequent stages of the analysis. Skin preparation, electrode selection and recorder attachment are important steps in the hook-up procedure. After the registration, sampling speed of the analog to digital (AD) conversion, the template correlation method and the analysis algorithm are points that should be carefully considered. Standard Holter equipment with a sampling frequency of 128 Hz is usually suited for the analysis of HRV, however higher sampling speeds are preferable[163]. Especially in patients with low HRV such as congestive heart failure or cardiac transplant patients, the relative contribution of the measurement error due to sampling speed can increase significantly[3], since in these patients the measurement error due to the sampling speed is high compared to the amount of true variability. Recorders and analysis systems that do no utilize a correction system for irregular recordings should not be

used for the analysis of HRV. The technical aspects of ECG registration and analysis are discussed in more detail in chapter 3.

2.4.2 ECG analysis

Independent of the type of equipment used, the analysis of ambulatory monitoring recordings is quite uniform. During ECG preprocessesing by the analysis equipment, the so-called stand-alone phase, a number of ECG analysis steps can be distinguished. Of these, the three most important steps are:

1. QRS detection - the detection of peaks in the ECG signal
2. Noise rejection - the detection and removal of noisy data
3. QRS classification - the forming of templates

All of these steps may have a profound influence on the outcome of HRV analysis and therefore deserve close attention. This chapter provides an overview of the different ECG analysis steps and their impact on the outcome of HRV analysis.

QRS detection:
It is of great importance that QRS complexes are detected at the same point (QRS onset or QRS peak). Besides the technical measurement error of the AD conversion (chapter 3.1.2), an error may also be introduced by the analysis system during the QRS detection process. This is especially true in the presence of wide QRS complexes, i.e. intraventricular conduction disturbances. If all QRS complexes would be triggered too early or too late by the algorithm, the outcome of HRV analysis would not be influenced. However, usually some QRS complexes are correctly detected while other QRS complexes are detected relatively early or late. This may result in timing differences of up to 150 ms, especially in patients with wide QRS complexes. As a consequence, an unpredictable change in HRV variables may occur. HRV variables averaged over 24 hours are influenced to a lesser extent than variables computed over short segments. Also, HRV variables reflecting relatively fast changes in heart rate are affected more than variables reflecting slower variations. A technically more detailed description can be found in chapter 3.2.2. For reliable HRV analysis, proper detection of QRS complexes is mandatory. If consistent QRS detection of an ECG cannot be guaranteed, HRV analysis should not be performed.

Non sinus intervals during sinus rhythm:
Ambulatory monitoring recordings suffer from different sources of noise. Some of these, such as loose electrodes make proper analysis of ECG impossible. Also muscle and movement artefact may cause inaccurate analysis of the ECG. Since it is practically impossible to manually review and label every QRS complex, the quality of the recording and hence, the amount of data that is

excluded will influence the outcome of HRV analysis. These episodes of disturbed ECG consist of noise and episodes with frequent ectopic activity. These two sources of disturbance result in episodes of so called non-sinus intervals (NSI). The amount of NSI that is present in clinical quality Holter recordings is shown in Figure 12, expressed as a percentage of total recording time. Ways to improve the quality of ECG registrations are described in chapter 3.

QRS classification and ectopy exclusion:
After detection, QRS complexes are automatically divided into templates - i.e. groups containing look-alike beats - by the Holter analysis machine. These templates are visually checked for consistency and correct labelling by an analyst. During this process the analyst discriminates between sinus rhythm and the various forms of ectopic beats. Since the autonomic nervous system mainly influences the sinus rhythm, intervals that are initiated and ended by a beat originating from the sinus node are the only type of intervals that should be included in the analysis of HRV. Proper identification of NSI is therefore mandatory. Exclusion of NSI will lead to a sequence of "normal to normal intervals" or NN-intervals which can be used for further analysis. Unfortunately, perfect detection of NSI is not always possible, especially in patient populations with frequent multiform ventricular arrhythmias. Ectopic beats may or may not cause a reset of the sinus node, leading to a disturbance of the subsequent (sinus)rhythm. The influence of ectopic beats has been the subject of a number of studies[28, 42, 253].

Excluding segments with frequent ectopics may also lead to a selection bias[131, 132], since increased prevalence of ectopy may be the cause of changes in the output of the autonomic nervous system. Although limits with respect to the amount of NSI that may be accepted for analysis cannot be given, in our opinion, data segments containing more than 15% of time-based NSI, should always be excluded from HRV analysis. Data segments containing less than 5% of time-based NSI, may be safely used for the analysis of HRV.

In several studies, the ectopic beats are excluded from the ECG using automatic filters. These so-called percentile exclusion rules exclude beats based on their timing compared to one or more preceding intervals. Although the guidelines clearly state that this may lead to unreliable results, these filters are frequently used. We investigated the effects of several percentile exclusion rules and found that time- as well as frequency-domain variables may be influenced significantly. Furthermore, the composition and prematurity of ectopic beats proved to be different for the populations that were studied, thus demonstrating theoretical as well as practical arguments against the use of percentile exclusion rules. Manual editing of the ECG is mandatory. When manual editing is performed accurately, the addition of a percentile exclusion rule has no function. A detailed description of the influence of ectopic beats is provided in chapter 3.2.3

2.4.3 HRV analysis methods

HRV can be determined in many ways, quantitative as well as qualitative (descriptive). In order to differentiate between the various types of variations in heart rate, different HRV methods and variables have been developed. A flow chart of HRV analysis, depicting the different preparing steps and HRV analysis methods is presented in Figure 2. This thesis focuses on linear techniques, however non-linear techniques are very promising especially when a complex underlying mechanism is assumed, for example in advanced stages of congestive heart failure or during atrial fibrillation.

Graphical presentations:
Physicians often use, without realising it, graphical presentations that contain information about the variations in heart rate, which can be considered a basic form of HRV. Such graphical presentations are present in virtually all diagnostic and monitoring devices within the field of cardiology. Two types of graphical presentations particularly stand out: heart rate trends and histograms.

Heart rate trends:
The most basic and well known graphic form that provides insight in the distribution of RR-intervals is the heart rate trend. One advantage of this representation is its simplicity, i.e. the heart rate trend can be reviewed at one glance. In HRV three aspects can be used to study RR-intervals:
1. the magnitude of RR-intervals
2. the relation of an interval towards other intervals and
3. the time scale.

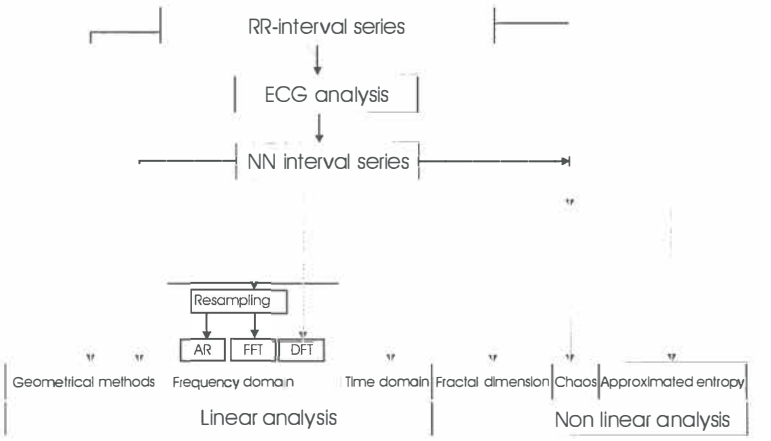


Figure 2. HRV flow chart. This figure shows the preparing steps as well as the different techniques of HRV analysis in sequence, divided into linear and non-linear analysis techniques.

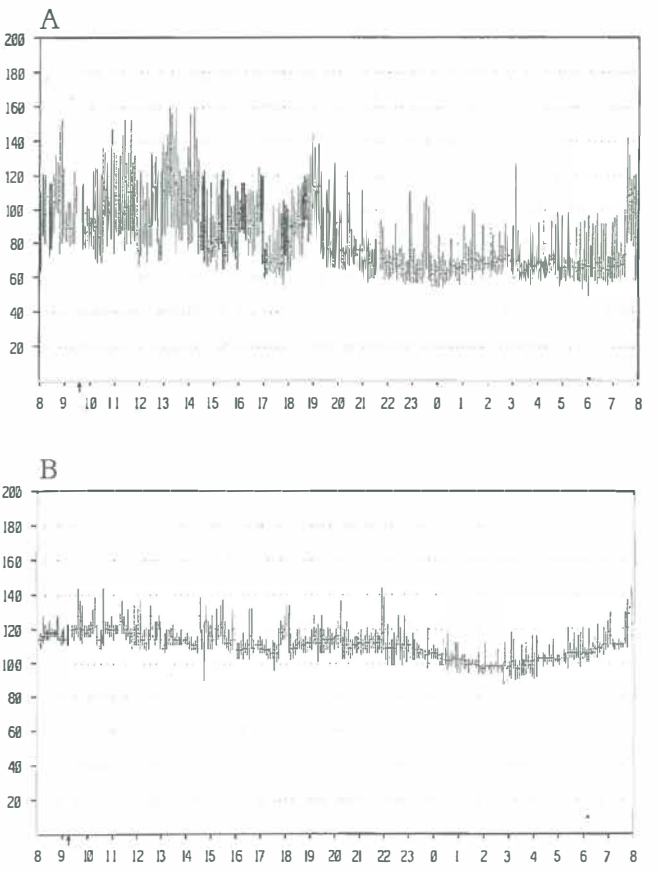


Figure 3.
Heart rate trend of normal (A) and depressed (B) HRV. Average heart rate is increased, while band width and day night difference are markedly reduced in the panel B.

To a certain extent, all 3 aspects of HRV are present. As an example two heart rate trends are shown in Figure 3. In panel A the heart rate trend of a healthy subject is shown, the heart rate trend in panel B was obtained from a patient with congestive heart failure.

Three aspects of the heart rate trends stand out:

1. The average heart rate of the trend in panel B is higher.
2. The bandwidth, i.e. short-term variability in HRV, of the trend is lower in panel B.
3. The day-night difference of the trend is reduced in panel B.

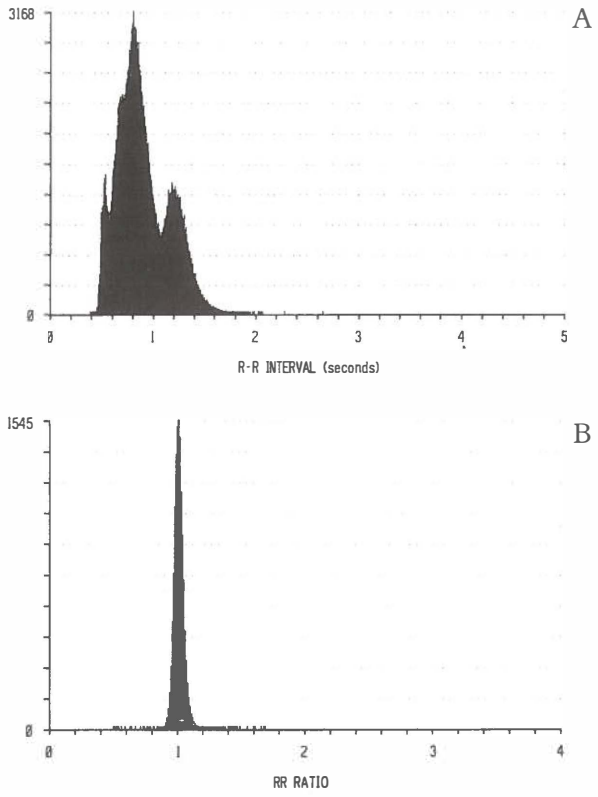


Figure 4.
RR-interval histogram (panel A) and RR-ratio histogram (panel B) in a healthy individual. The RR-interval histogram shows a day and a night peak, while a ratio of 1 indicates a regular rhythm.

Histograms:

A RR-interval histogram (Figure 4, panel A) is a graphical presentation of the distribution of all RR-intervals. In such a histogram, the length of an RR-interval is shown on the X axis and the frequency count observed in a recording is shown on the Y-axis. A histogram that shows the relation with other intervals is the so-called RR-ratio histogram (panel B). In this histogram, the ratio between each RR-interval and the preceding interval is plotted. During a strictly regular sinus rhythm with equal intervals this ratio will be 1. However, occurrence of premature beats result in a ratio of less than 1. A drawback of this representation is that it contains neither the absolute size of the RR-intervals nor a time-scale. The width of a RR-ratio histogram is a measure of beat-to-beat fluctuations. Indeed, the width of such a histogram is a measure of HRV. RR-interval histograms are graphical tools that show what happens, not when it occurs. The distribution of RR-intervals may show specific patterns. In Figure 4, panel A, the RR-interval histogram shows a typical distribution of RR-intervals, with a clear short and long interval peak, representing the day and night RR-interval difference, respectively. From the RR-interval histogram, two geometric variables of HRV can be derived: the triangular index and the TINN. These measures are explained in chapter 4.1.3.

Time-domain analysis

Time-domain analysis of HRV is a method that uses relatively simple mathematical measures of RR-intervals to determine the amount of variation in a series of RR-intervals. In the field of cardiology, the first study that drew clinicians attention to HRV was the article by Kleiger et al.[122]. This study showed an increased risk for overall mortality after myocardial infarction in a group with a low SDNN (< 50 ms) when compared to patients with a high SDNN (> 100 ms). Time-domain variables can be divided into overall measures of HRV (AVGNN and SDNN), measures of long-term fluctuations (SDANN), intermediate variables (SDNN index) and variables representing short-term or beat-to-beat changes (rMSSD and pNN50). A detailed description of the different variables and their characteristics is given in chapter 4.1.1. A major advantage of time-domain analysis is the simple and robust method by which the variables are computed. Time-domain variables can be computed easily and there is consensus how to compute these variables[2]. Time-domain variables are dependent on heart rate and age (see chapter 5). Therefore, when comparing groups, these groups should be matched with regard to age and heart rate. As discussed in chapter 2.4.2 and 3.2.3, thorough editing of the ECG is mandatory in the process of time-domain HRV analysis. Automatic exclusion of ectopic beats and noisy ECG is insufficient to obtain reliable results. Furthermore, time-domain variables are sensitive to changes in recording duration as discussed in chapter 6. Therefore, in any study at least the average duration of recordings should be reported for each group. An advantage of time-domain variables in risk stratification is their stability over time[102, 184, 267]. Time-domain variables may be used to identify patients at risk with several disorders such as unstable angina pectoris[101], myocardial infarction[65, 122, 234], and diabetes mellitus[230].

Frequency-domain analysis

The essence of frequency-domain analysis is decomposition of the heart rate signal into its constituent components. As a prism decomposes light, frequency-domain analysis disentangles an RR-interval series into its various components (Figure 5). The result of frequency-domain analysis is a so-called spectrum. Depending on the duration of a data segment that is used for the calculation of such a spectrum, 4 (in case of a full 24-hour spectrum) or 3 (in case of 5-minute segment spectra) frequency bands are usually distinguished :

1. Ultra low frequency power: ULF (< 0.0033 Hz)
2. Very low frequency power: VLF (0.0033 - 0.04 Hz)
3. Low frequency power: LF (0.04 - 0.15 Hz)
4. High frequency power: HF (0.15 - 0.40 Hz)

Using 5-minute spectra, the lowest available frequency is 1 / 300 sec = 0.0033 Hz, which is the lower boundary for VLF frequency. This implicates that ULF power can only be calculated using long-term recordings.

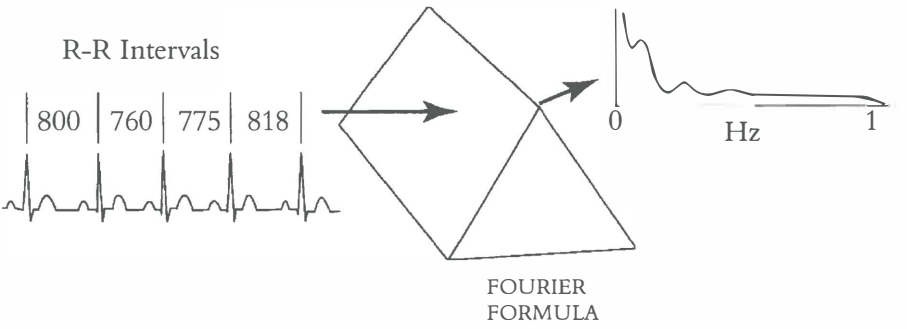


Figure 5. Frequency-domain analysis: The effect of (fast) Fourier analysis (FFT) is the decomposition of a signal into the various frequency components. As a prism decomposes light into the different components, the FFT is able to decompose a signal into the various frequency components that form the total signal.

The existence of frequency bands and their boundaries have been the subject of a number of investigations[5, 91, 212]. Although fixed boundaries have no unequivocal physiological basis, fixed frequency bands are used. A drawback of using fixed boundaries is the fact that high respiratory rates may shift the HF peak partially outside the observed range, for example in young children. This drawback is to be weighed against the advantages of standardisation. All frequency boundaries mentioned here are in accordance with the proposed boundaries as defined in the guidelines[2].

An example of a spectrum and the frequency bands is shown in Figure 6. The HF peak, centred at approximately 0.25 Hz. is caused by the well know respiratory sinus arrhythmia (15 breaths per minute = 0.25 Hz). Obviously, 15 breaths per minute equals 1 per 4 seconds, which is 0.25 cycles per second, or 0.25 Hz. These fluctuations are predominantly vagally mediated[5, 152, 195] while the origin of the LF peak is less clear. Some investigators attribute the LF peak entirely to sympathetic influence[152, 172] while others consider it to reflect a combination of vagal and sympathetic influences[5]. The variables mentioned above are referred to as absolute measures of frequency-domain HRV[2, 189] The most frequently used and generally accepted frequency-domain variables are described in detail in chapter 4.1.2. Compared to time-domain analysis, frequency-domain analysis is a computationally more elaborate method and moreover, more difficult to understand and less robust. Obviously, a relationship exists between time- and frequency-domain variables of HRV. This was described by Bigger et al[22]. Time- and frequency-domain variables measuring fast fluctuations in RR-intervals, such as rMSSD and HF, show a high level of correlation, since they measure the same underlying phenomenon and because

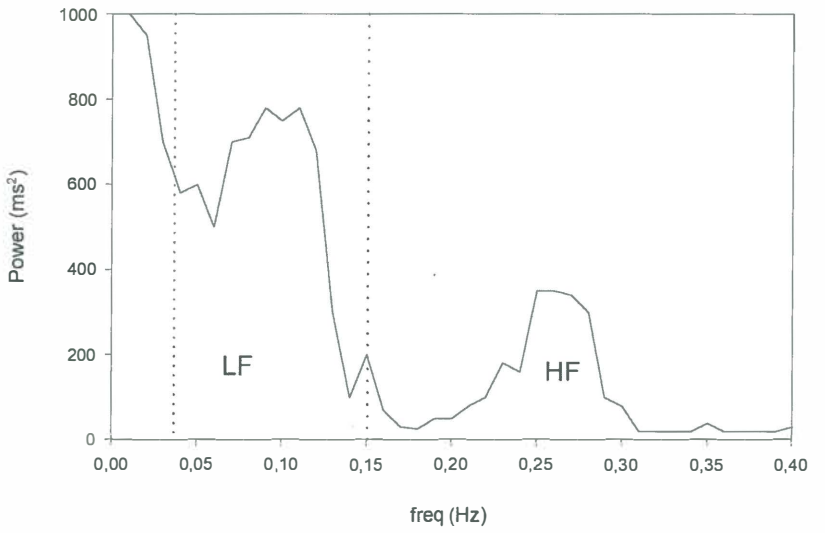


Figure 6. Example of a power spectrum computed from a 5-minute segment. The power spectrum shows the decomposition of the original RR-interval signal into the various frequency components, depicted here as high and low frequency power.

a mathematical relation exists. This is also true for HRV variables measuring slow fluctuations. Furthermore, the power in the time domain or variance, equals the overall power in frequency domain. This is expressed by Parseval's Theorem (Equation 1). In time domain the power of the signal (*h(t)* in equation 1) consists of the average interval (the DC component) combined with the variations around this mean (the AC component). The variations around the average are mathematically expressed by the standarddeviation. Before frequency domain analysis is applied the average is usually subtracted, therefore the signal input of the frequency domain analysis is the AC component only. In equation 1 *H(f)* represents the frequency equivalent of *h(t)*.

In simple terms this means that ideally the square of the SDNN equals the overall power obtained by means of frequency-domain analysis. Although frequency-domain variables are more difficult to compute compared to time-domain variables and strong correlations do exist, frequency-domain variables should not be discarded. Time-domain variables such as SDNN often cannot be used to adequately characterize fluctuations in the physiological system. In these instances frequency-domain analysis is more suitable. Inspection of a spectrum can be very helpful in understanding what has actually happened. For example, a shift in breathing frequency would be easily visible in a spectrum, while this is more difficult to detect using time-domain analysis.

$$\int_{-\infty}^{\infty} |h(t)|^2 dt = \int_{-\infty}^{\infty} |H(f)|^2 df$$

Equation 1. Parseval's Theorem: power in time domain equals power in frequency domain.

Frequency-domain analysis over sequential 5-minute segments:
Ideally, ambulatory monitoring recordings are 24 hours in duration. In HRV analysis, these recordings are analysed as a whole, or in smaller segments with a duration of for example 5 minutes. Although 24-hours analysis in toto allows for the computation of VLF and ULF, the analysis of repetitive short segment is often preferred. This preference is due to the fact that short-segment analysis allows for exclusion of episodes with frequent NSI and takes considerably less computational time. With respect to frequency-domain variables LF and HF, Rottman et al.[2,107] showed that the outcome is the same using the in toto method or consecutive 5-minute analysis.

According to the guidelines[2], ECG recordings should last for at least 10 times the wavelength of the lower frequency bound of an investigated component and for stability reasons should not last much longer. However, in the same article short data segments of 2 minutes are accepted without further elucidation. The consequence of this rule is that the minimal segment of ECG that is required to compute LF power is 250 seconds (since the lower bound of LF is 0.04 Hz., corresponding to 25 seconds.) Therefore, only data segments of approximately 5 minutes are suitable for calculation of the LF component. Technically, VLF can also be obtained from 5-minute segments. However, to reliably measure this variable, longer data segments are necessary. If one, besides LF, also wants to calculate the HF measure, required to get the full context in spectral HRV analysis, the same data segment should, according to this rule, not exceed 66 seconds (10 times the lower boundary of HF which is 0.15 Hz corresponding with 1 / 0.15 = 6.66 seconds). Therefore, for practical reasons the guidelines recommend the use of 5-minute segments. In this respect Akselrod and Keselbrener[117] demonstrated a very interesting possibility. They use different data segment durations for each specific spectral component, for low frequencies a longer and for high frequencies a shorter data segment. The authors were thus able to detect changes in frequency location and amplitude of the respiration peak during a controlled respiration experiment, the effect of positional change (stand-up test) and short episodes of transient changes in autonomic influences that could not be detected using standard Fourier techniques[118]. This new technique has not gained wide acceptance yet, but certainly deserves attention. In literature, analysis of 5-minute segments averaged to a 24-hour mean is the most frequently used technique.

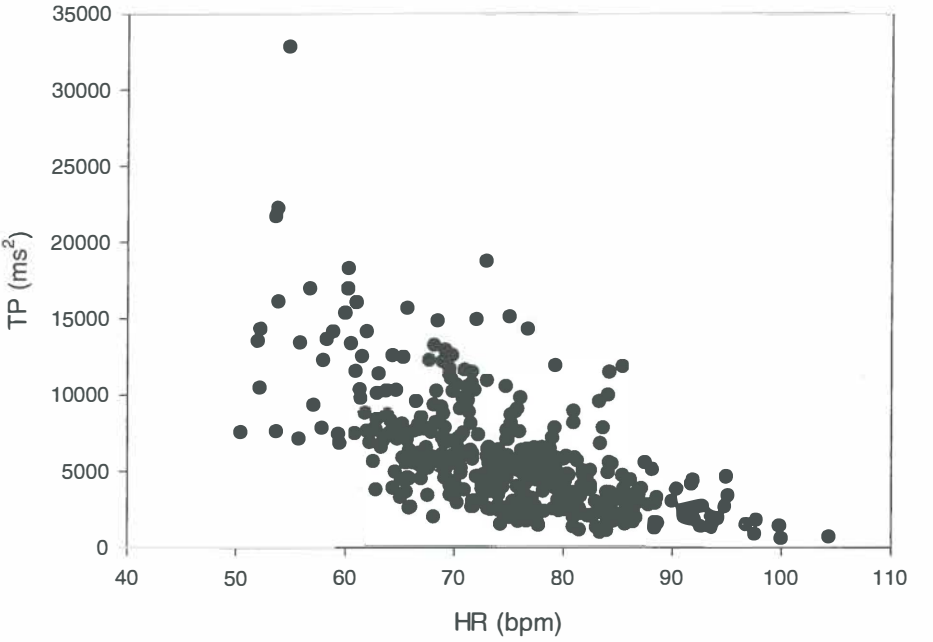


Figure 7. Relationship between TP and average heart rate in the recording in 419 healthy subjects. This figure clearly demonstrates a correlation between the decrease in total frequency power (TP), computed by means of frequency-domain analysis, and the increase in heart rate.

Normalisation

Average heart rate (or its inverse AVGNN) can be considered a global (but not necessarily the most sensitive) HRV variable. A shift in sympathovagal balance towards sympathetic domination causes heart rate to increase and all the previously described frequency-domain variables to decrease. As an example, TP shows a clear relation with heart rate (Figure 7). This implies that the comparison of signals with a different heart rate is troublesome. Although mathematically the average of a signal and its variance are independent measures, heart rate and HRV variables both depend on the influence of the autonomic nervous system and are therefore not independent. In the frequency domain, three methods are used to study relative shifts of HRV instead of absolute shifts: normalized units, component coefficient of variance and ratio of LF to HF power (or LFHF ratio)

Normalized units:

The first and most frequently applied correction method is that of calculating the "normalized units", i.e. LFnu and HFnu. As stated in the guidelines:

"normalisation tends to minimize the effect of the changes in TP on the values of LF and HF components". The normalized values of LF and HF are calculated by dividing the absolute power of one component by the sum of both.

Therefore, HFnu = $\frac{HF}{HF + LF}$ and LFnu = $\frac{LF}{HF + LF}$. Because of the fact that a substantial amount of power lies below 0.04 Hz, this normalisation procedure only partly corrects for differences in heart rate. Normalized units merely show the balance between the vagally mediated and sympathetically dominated component of the power spectrum. LFnu is suggested to be a superior marker of sympathetic activity compared to LF expressed in absolute units[150]. Considering relative as opposed to absolute values is one of the points that has led to misinterpretation of data. Decrease of LF expressed in absolute units has often been interpreted as a decrease of sympathetic tone. Besides the fact that HRV variables only reflect variations in tone, a change in any frequency band must be seen in the context of total power, and therefore always be expressed in absolute as well as normalized units[151].

Coefficient of component variance: A better method of correcting for differences in heart rate is dividing the absolute units by the AVGNN. In a study assessing the relationship with cardiac vagal tone, Hayano and co-workers called this correction the coefficient of component variance (CCV variables). In their study, an excellent correlation was shown between cardiac vagal tone on the one hand and CCV variables of HF power on the other. Chapter 8 contains an in-depth technical description of this effect.

LFHF ratio: This variable has a strong computational relation with the normalized units discussed above, and is also not suited for correction of differences in heart rate either.

Like time-domain variables, frequency-domain variables are affected by heart rate, recording duration and errors in beat detection and classification. Frequency-domain variables can be applied to identify high-risk patients with congestive heart failure[174], myocardial infarction[103], diabetes mellitus[4] and hypertrophic cardiomyopathy[51]. Furthermore, in a community based population, frequency-domain variables of HRV were found to be related to all-cause mortality[234].

Reproducibility of time- and frequency-domain variables

Reproducibility of time- and frequency-domain HRV analysis, computed over 24-hour ambulatory monitoring recordings has been the subject of a large number of investigations, and was shown to be one of the strong points of this analysis method. Most other variables that are used to predict mortality, such as New York Heart Association classification, left ventricular ejection fraction, the result of exercise testing, silent ischaemia and ventricular arrhythmias may

vary over time. In contrast, HRV has been reported to be remarkably stable over time in ambulatory monitoring recordings for a number of populations:

1. ***Healthy individuals*** - rMSSD on two consecutive days showed no significant difference[267]. For SDANN, a low intra-subject variability was found using 2 ambulatory monitoring recordings over a time period of 2 - 7 days[102]. Time- and frequency-domain variables were also shown to be stable over a period of 3 - 65 days and placebo effect was absent[121] as well. In another study assessing a large group of healthy individuals (n=173), virtually identical results were obtained from two ambulatory monitoring recordings using clinical quality ambulatory monitoring recordings that were made 13 ± 9 days apart[184]. Only one single study by Van Hoogenhuyze et al.[248] showed a marked intra-individual (day to day) variation for SDNN, SDANN and CV, although good reproducibility for the group mean value was found. The authors do not speculate on the cause of this relatively poor reproducibility compared to other studies, nor do the methods of the study present any apparent information. In 17 healthy individuals and 20 patients with myocardial ischaemia, highly reproducible results were found[99] when comparing HRV results from ambulatory monitoring on day 1, 7 and 28.
2. ***Ischaemic heart disease*** - In 33 post-MI patients, Kautzner et al [116] showed good reproducibility for the variables SDNN, SDANN, SDNNindex and rMSSD. For SDNN and SDANN the day to day reproducibility was 8.5 ± 7.3% and 13.8 ± 13.5% respectively. Multiple studies investigating ischaemic patients showed no significant variation between two consecutive recordings [19, 110, 191], over a 7-day period[124] and over a period of 14 ± 13 days [184]. Pardo et al[191] found good reproducibility measured from 2 consecutive ambulatory monitoring recordings, despite varying severity of ischaemia over these days.
3. ***Congestive heart failure*** - Again a good group mean reproducibility, with significant intra-individual (day to day) variation was shown by Van Hoogenhuyze et al[248]. Ponikowski et al [196] reported poor reproducibility, however, this analysis was performed using single measurements over a range of relatively short data segments. Analysis of 24-hour ambulatory monitoring recordings, recorded 2 weeks apart was performed by Stein[224] and revealed a good reproducibility.
4. ***Diabetes mellitus patients*** - A study by Nolan[184] showed stable behaviour of HRV in this patient category measured over 1,2,3 and four months.

Using short segments of data Breuer et al[37] found a poor reproducibility of the expiratory / inspiratory ratio of heart rate. They concluded that the poor reproducibility does not permit a reliable interpretation of HRV on the basis of single measurements in healthy volunteers. Toyry and co-worker also found a relatively high variability of rMSSD in healthy subject measured over 1 minute

of fixed breathing. These authors concluded that: "One-minute fixed pace breathing period seems to be too short to allow reproducible measurement of rMSSD and the frequency-domain variables of heart rate variation." For time-domain variables computed over 24 hours, all studies, except one[248] report excellent reproducibility in the previously mentioned patient categories. This facilitates the practical use of HRV.

In conclusion, reproducibility of both time- and frequency-domain HRV measurements is very good, when based on 24 hours. Analysis of single, randomly taken 5-minute segments may show considerable fluctuations and should therefore not be used as a global measure of HRV.

2.4.4 Normal values

Before a certain measure of HRV can be considered abnormal, normal values have to be established. HRV is known to decrease with age[52, 130, 193, 202] and is also said to be dependent on gender[208], although this is stated by others to be the result of differences in heart rate. Unfortunately, analysis of large populations is limited to short-term recordings[233] or evaluating time-domain variables only[202, 239]. Only one relatively large study was performed using time- as well as frequency-domain variables in a younger group[160] (age 3 days to 14 years). The guidelines[2] present only limited normal values, but emphasize the need for age and gender corrected normal values. It is noteworthy that normal values of frequency-domain variables are dependent on a number of technical issues such as the amount of NSI accepted as well as the computational method. Therefore, normal values of frequency-domain variables can only be used when the methodology matches exactly. We investigated normal values of HRV, time- as well as frequency domain, and their dependence on heart rate, gender and age in a group of 419 healthy subjects. A significant relation was found between age and all variables of HRV. All HRV variables declined with age. In some variables the decline is especially strong in the early years of life, while in others the decline was more gradual. This finding is consistent with the findings of others[223, 239]. Also a significant relation was found with heart rate. Values of virtually all HRV variables decreased with increasing heart rate. Multivariate analysis revealed no relation between gender and HRV variables when heart rate was included in the analysis. In other words, gender-related changes can be explained by changes in heart rate. Figure 8 shows the relation between HRV, gender and age. The relation of SDNN with age first shows a rapid decay; however, this process slows considerably at higher ages. These mechanisms, and the normal values are described in detail in chapter 5.

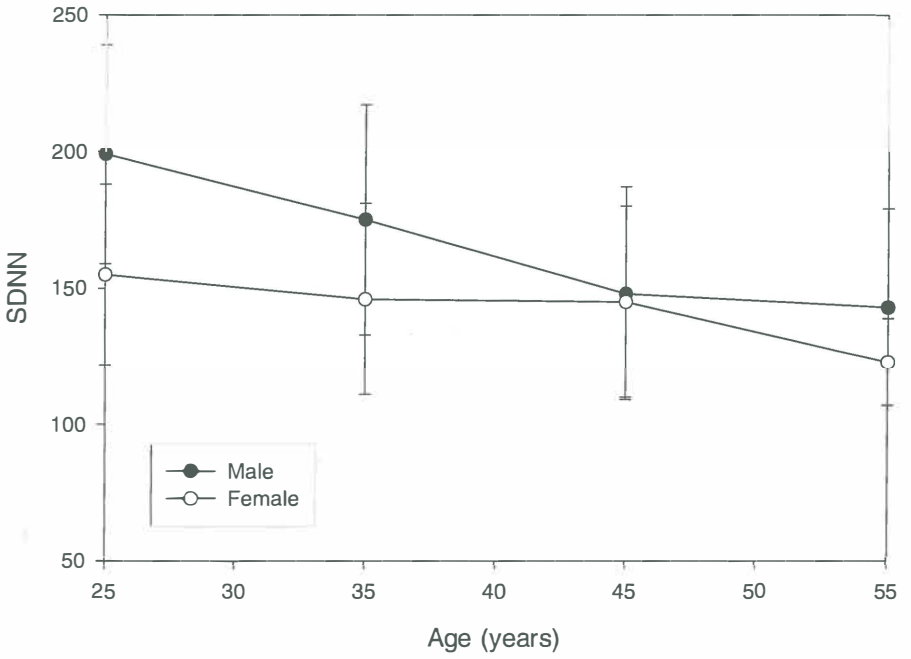


Figure 8. SDNN vs. AGE in a healthy population of 274 male and 118 Female subjects. The dependence of SDNN on age and gender. SDNN decreases with increasing age and is slightly lower in female subjects.

2.4.5 The influence of recording duration

Because of their mathematical properties, frequency-domain variables are influenced by recording duration. Furthermore, in clinical practice, hook-up times of ambulatory monitoring recordings are not randomly divided over time because the start of a Holter recording is usually during day hours. Ideally a Holter recording is 24 hours in duration. Therefore, the end-time of the Holter equals the hook-up time. However, often a Holter recording is ended prematurely. This means that recordings with a duration less than 24 hours will primarily miss the last hours of the recording, often "daytime hours" or hours in the early morning. Therefore, shorter recordings will often fail to record data dominated by the sympathetic part of the autonomic nervous system. The guidelines state that the minimal duration of an ambulatory monitoring recording should be 18 hours, containing at least the whole night. Although these statements are undoubtedly useful, little or no literature is available that assesses these matters. In a post-myocardial infarction group the predictive value of HRV obtained from 1-hour recordings was compared to HRV estimated from 24-hour recordings[145]. The authors concluded that arbitrarily chosen 1-hour segments cannot replace 24-hour average of HRV.

Furthermore, it is likely that the effect of recording duration is not similar for different groups of patients. If variability is low, also the changes in variability are probably less pronounced over time compared to normal variability. Therefore in patients with depressed HRV, duration-related changes will probably be less pronounced than in normal subjects. The effect of recording duration is shown in chapter 6. If recordings of different duration are compared, using the shortest duration as the standard for such comparison should be considered. For both time- and frequency-domain variables, recordings should not be less than 20 hours in duration[82].

2.4.6 Breathing

As was already explained in chapter 1, lung stretch receptors, the respiratory control centre and a number of other mechanisms cause beat-to-beat changes in heart rate often referred to as respiratory sinus arrhythmia. These rapid changes can be measured using the HF component of frequency-domain analysis. Studies by Pagani and Hayano have shown HF to be a marker of vagal activity[85, 189]. Brown and co-workers[41] have shown that breathing frequency and, to a much lesser extent, tidal volume influence LF and HF. Changing the breathing frequency caused the respiratory peak to move with the breathing frequency. In some studies the effect of a change of breathing frequency is compensated by recording the breathing pattern simultaneously and performing a corrected analysis form[9]. Obviously this is not feasible during 24-hour ambulatory ECG registrations. Metronome breathing is advocated to assess cardiac vagal control more accurately than free breathing. In some However, the additional value of metronome breathing over spontaneous breathing has never been established. Therefore, we investigated the effect of metronome breathing on HRV. In order to do so, we compared episodes of free breathing to episodes of metronome breathing, during the various stages leading to autonomic blockade. During metronome breathing, several variables showed a lower absolute value; nonetheless a strong correlation was found between variables computed during spontaneous breathing and metronome breathing. During metronome breathing a decrease in all HRV variables was observed when compared to spontaneous breathing except for HF (normalized units). This indicates a shift towards vagal predominance. The correlation of HRV variables with vagal activity during metronome breathing was somewhat better than during spontaneous breathing. We concluded therefore that metronome breathing has some advantages over spontaneous breathing in HRV measurements. However, using the commonly accepted frequency ranges, the differences are limited and therefore the effect of controlled breathing does not seem of great importance. Furthermore, metronome breathing is often experienced as unpleasant and stress-inducing. It may therefore even be considered contra-indicated when assessing autonomic control.

2.4.7 HRV in relation to disease

Analysis of HRV has been found useful in several cardiac as well as non-cardiac diseases. HRV has been shown to provide an easily obtainable early marker for progression of disease[237] According to the guidelines[2] two accepted indications exist for clinical use of HRV: post myocardial infarction and diabetes mellitus. In several studies, HRV has been shown to be a useful tool for risk stratification. However only limited data exist to show whether patients with low HRV actually improve after treatment. Furthermore increasing HRV through treatment does not necessarily imply improving the condition of the patient. Since the publication of the guidelines several articles have provided new data with respect to diseases and medication. This paragraph provides an overview of a number of relevant disorders and the behaviour of HRV.

Myocardial ischaemia

Myocardial ischaemia caused by to imbalance of oxygen supply and demand of the myocardium and may result in impairment of contractility and hence, myocardial function. As known from echocardiographic studies, wall motion disturbances are among the first signs of ischaemia. Impaired contractility may lead to lower cardiac output. As a result, activation of the baroreflex system may cause sympathetic activation and/or vagal withdrawal. Also, the direct influence of damage due to ischaemia or infarction may lead to impairment of autonomic control[149]. HRV has been shown to be impaired in both chronic and acute forms of myocardial ischaemia. In patients undergoing coronary angiography[88], a significant negative correlation was found between HF and the severity of coronary artery disease. In another study, without a change in heart rate, several measures of HRV were found to change shortly before transient episodes of myocardial ischaemia, while this phenomenon did not occur in control episodes without ischaemia[74]. In a similar study, a decrease in the HF component was observed shortly before an episode of myocardial ischaemia which persisted during the episode and recovered shortly after[249]. In a study comparing patients with stable and unstable angina pectoris[101], significant reductions of all time- and frequency-domain variables were found in both patient groups, when compared to control subjects. However, no differences between the two patient groups with angina pectoris could be demonstrated. In the unstable angina pectoris group, patients that stabilized showed a recovery in HRV variables, whereas this was not observed in patients with persisting complaints or persisting ischaemia. Also, in patients with unstable angina pectoris and transient episodes of myocardial ischaemia, time-domain variables were shown to be lower, compared to patients without myocardial ischaemia. Finally, patients with subsequent events (death or non-fatal myocardial infarction), had lower HRV values, compared to patients without subsequent events. In a large group of patients with coronary artery disease, only those

with episodes of ischaemia and non-sustained ventricular tachycardia showed low values of vagally related variables of HRV[241]. Finally in patients with syndrome X, an inverse relationship was shown between perfusion heterogeneity and variables of HRV[161]. In this study, a relationship was suggested between impaired myocardial flow and impaired autonomic control.

Conclusion: HRV is in general related to the severity of coronary artery disease and also to the occurrence of transient episodes of ischaemia. The limited data available suggest that HRV may identify patients at risk for subsequent events or cardiac death. Further studies are necessary to elucidate the full potential of HRV analysis in these patients.

Myocardial infarction

Like myocardial ischaemia, myocardial infarction may lead to changes in contractility and thus set the stage for the process already described, which is responsible for the observation that HRV is decreased after myocardial infarction. Although this is often not appreciated: "Predischarge 24-hour mean heart rate is a strong predictor of mortality after myocardial infarction that can compete with left ventricular ejection fraction and HRV"[47]. This has also been shown in other studies[93, 141].

Wolf et al[261] were the first to describe a reduced sinus arrhythmia in post myocardial infarction patients. Another pivotal study was that by Kleiger et al[122]. In post myocardial infarction patients they identified patients at risk using only one simple time-domain variable of HRV, i.e. SDNN. During follow-up, the relative risk of mortality was 5.3 times higher in the group with SDNN < 50 ms compared to the group with SDNN > 100 ms. In subsequent studies, also frequency-domain variables, especially ULF and VLF, but also LF and HF were shown to be independent predictors of mortality[23]. In the latter study the association between VLF power and arrhythmic death was stronger than with cardiac or all-cause death. Adding variables of HRV to previously known post-infarction risk predictors enhanced identification of small subgroups of patients with a 2.5-year mortality risk of approximately 50%. Shortly after myocardial infarction, frequency-domain variables are significantly reduced compared to age- and gender-matched controls; however, these variables tend to recover during the first three months after myocardial infarction. Thereafter, they remain stable up to at least 12 months post myocardial infarction[21, 138]. Frequency variables obtained late after myocardial infarction also predict death[20]. Furthermore, low HRV after myocardial infarction has shown to be predictive for early as well as late arrhythmic events and sudden death[54, 64, 188]. Compared to left ventricular ejection fraction, Odemuyiwa et al, found HRV to have higher sensitivity and specificity[187] for the predication of all-cause mortality, arrhythmic events and sudden death. In an extensive review, particularly variables representing slow HR variations had a univariate and strong relation with death[22]. In a post-myocardial infarction study in 80 patients using

echocardiography, a low HRV group with a triangular index <= 25 showed an increase in end systolic volume and end diastolic volume, while the high HRV group remained unchanged after 12 months (for explanation of this variable see chapter 4.1.3). Assessment of the HRV index before discharge, proved to be an independent risk factor for left ventricular dilatation[56]. HRV was found to be reduced in patients in whom ventricular tachycardia could be induced, compared to patients that were not inducible during electrical stimulation. In this respect reduced VLF proved to be the strongest independent predictor of VT susceptibility[103].

Conclusion: HRV can be used to identify patients at risk for cardiac events after myocardial infarction such as arrhythmic events, re-infarction and death. Although HRV is impaired after myocardial infarction, it usually shows a gradual recovery. However, HRV is found to have a predictive up to 1 year after myocardial infarction. Therefore the application of HRV for the purpose of risk stratification is feasible in clinical practice.

Congestive heart failure

Congestive heart failure is a syndrome in which the heart fails to fulfil the circulatory requirements of the tissues and is usually accompanied by increased filling pressures. Associated activation of neuroendocrine systems is deleterious since it leads to progression of the disease. Depressed HRV in patients with congestive heart failure has been demonstrated in a number of studies[44, 211] as has a relation between markers of severity of disease and HRV[119]. Furthermore, a significant relation has been demonstrated between the extent of left ventricular dysfunction and variables of HRV representing vagal activity. Though frequent occurrence of ectopic beats in this patient population may hamper reliable measurement of HRV, especially in frequency-domain, the variations in sinus rhythm were shown to be unaltered immediately before and after premature ventricular complexes. From these observations, Woo et al concluded that time-domain variables such as SDNN may be reliably determined[263]. In contrast, Meyers et al showed that frequent occurrence of ectopic beats may result in unreliable power spectra[180]. Using HRV, high-risk subgroups may be defined. In a patient group awaiting cardiac transplantation, SDANN was found to be the variable with the greatest sensitivity (90%) and specificity (91%)[25] for mortality. Patients with SDANN values of <55 ms had a twenty-fold increased risk of death. These authors also conclude that HRV measurements are superior to other prognostic markers such as left ventricular ejection fraction, pulmonary artery wedge pressure, cardiac index, and serum sodium levels in this specific patient group. The underlying mechanisms that cause these HRV changes are supposed to be vagal withdrawal as well as sympathetic activation[27, 174]. Several variables of HRV have proven to be useful[39, 265] in predicting risk for (all-cause) sudden death. Especially the use of Poincaré plots may be useful in this context. Furthermore effects of medication could be demonstrated[238, 266].

Conclusion: The above findings indicate that HRV can play an important role in risk stratification and the evaluation of treatment in patients with heart failure and may therefore be used for these purposes in clinical practice.

Diabetes mellitus

In diabetes mellitus, autonomic neuropathy is a well-known complication. This phenomenon may be difficult to detect in early stages of disease due to compensatory mechanisms. In theory, there are three points at which diabetes mellitus may cause damage, resulting in impaired HRV. First of all the registration of signals may not be picked up and sent through to the brain. Secondly signals from the brain may not reach the heart due to damage of efferent nerves and finally, impaired responsiveness of the heart may cause a diminished sensitivity to signals of the autonomic nervous system. Ewing et al.[61] reported higher heart rates for patients with diabetes mellitus with vagal damage alone, compared to patients without vagal damage, but also compared to subjects with combined sympathetic and vagal damage. Because of their observation that sympathetic damage does not occur without vagal damage, they conclude that vagal damage occurs before sympathetic damage. The first investigations towards the practical use of HRV in patients with diabetes mellitus evaluated only simple HRV variables during short segments, and concluded that HRV was of limited or no use[15, 18]. In more recent and larger studies however, a strong correlation of the HRV response to deep breathing with the duration of diabetes mellitus was found[186]. Reproducibility of HRV was assessed in 87 patients with diabetes mellitus with long-standing disease and was found to be stable over a period of 1 - 4 months. A group of insulin-dependent patients with diabetes mellitus was compared to normal age- sex- and bloodpressure-matched controls. Frequency-domain analysis computed over short episodes was found to discriminate between the two groups, while more conventional tests of autonomic function failed to show difference[258]. In another study, 25 patients with insulin-dependent diabetes mellitus were divided into 2 groups, based on the result of standard tests of autonomic function. These standard tests (Vasalva manoeuvre and deep breathing) failed to demonstrate a significant difference between normal subjects and the patient group showing the least abnormalities. However, the standard deviation of RR-intervals was able to discriminate between the groups[154]. A study comparing children with diabetes mellitus and poor metabolic control to children with adequate metabolic control showed substantial differences between these groups using both time- and frequency variables [4]. Takase and co-workers[230] was able to discriminate using HRV between normal subjects, a group of diabetic patients with autonomic neuropathy and a group of diabetic patients without neuropathy.

Conclusion: HRV is a useful marker of autonomic status in patients with diabetes mellitus, distinguishing between patients and normals, disease state and level of metabolic control. This enables early detection of abnormalities

or worsening of clinical state and makes HRV a clinically useful tool in diabetes mellitus.

Hypertension

Essential hypertension is usually defined as a diastolic blood pressure consistently over 90 mm Hg without apparent explanation. Possible mechanisms that may play a role are the condition of the kidneys; nervous system, or blood vessels; various hormone levels in the body, stress and many more. As a consequence, the baroreceptor gain may decrease (less sensitivity) which in turn leads to vagal withdrawal and sympathetic stimulation. In a comparison between 40 hypertensive and 35 normal subjects, normalized LF power, LFnu, was found to be higher and HFnu was found to be lower in the hypertensive group. in this study a reduced response to passive head up tilt testing was observed as well[78]. In a study investigating the relationship between left ventricular mass and variables of HRV, a weak but significant negative relation was found between left ventricular mass and LF[125]. From this study, it was concluded that the level of end-organ damage correlates with neuronal alteration in essential hypertension; this was confirmed in another study[192]. A study of 168 long-standing hypertensive patients[105] showed lower time- as well as frequency-domain variables compared to normotensive subjects. It was concluded that long-standing hypertension leads to a reduced overall heart rate variability and results in blunted autonomic responses to changes in body posture. The authors also conclude that elevated blood pressure and obesity are the main causes of disturbed autonomic modulation of heart rate in males with long-standing hypertension as compared to normotensive subjects.

Conclusion: Small but significant changes in HRV are found in patients with hypertension. No data are available on the relation between severity of hypertension and the variables of HRV. Data is also lacking with regard to treatment effects. Further investigation is needed to assess whether HRV can play a role in clinical follow-up or treatment of hypertensive patients.

Amyloidosis

Amyloidosis is a syndrome characterized by the deposition amyloid fibrils in various tissues of the body. Depositions of amyloid do occur in the heart muscle cells as well as in the vagal and sympathetic nerve cells. This may lead to impairment of cardiac function and autonomic dysfunction. HRV analysis of patients suffering from amyloidosis often shows extremely decreased values of high- as well as low-frequency HRV variables. Until now, only a few small studies on the effect of amyloidosis on HRV have been conducted. Niklasson[183] showed that HRV was significantly depressed in a group of 12 patients compared to healthy individuals. Furthermore a relation was found between HRV and neurological disability. Using Poincaré plots and frequency-domain analysis, Suhr et al [226] were able to identify patients with familial amyloidotic neuropathy,

who were at risk for circulatory instability during subsequent liver transplantation.

Conclusion: HRV is diminished in patients with amyloidosis and is related to the extent of neuropathy. More large-scale studies are needed with respect to the clinical application of HRV in patients with amyloidosis.

From the diseases described above and their impact on HRV, it can be concluded that the decrease of HRV is related to the severity of both cardiac as well as non-cardiac disorders. The mechanisms by which this decrease is caused may be divided into two groups: first, a group in which damage to the heart itself causes impaired function (e.g. ischaemia, myocardial infarction and congestive heart failure); secondly, a group of disorders in which also the afferent and efferent nerves are damaged (e.g. diabetes mellitus and amyloidosis). Such a difference in mechanism may potentially allow for the distinction between several disorders based on a specific HRV pattern. HRV is a non-invasive tool for risk stratification, that can be performed using routine clinical ambulatory monitoring.

2.4.8 The relation of HRV and medication

As discussed previously, after myocardial infarction, depressed HRV is related to poor clinical outcome and increased mortality risk. It should be noted however, that this does not mean that improvement of HRV in itself leads to better clinical outcome or lower mortality. Only limited data are available on beneficial effects of (pharmacological) treatment of patients in relation to improvement of HRV. Pharmacological interventions may influence among other things blood-pressure, contraction strength and heart rate. The baroreflex mechanism reacts to changes in blood-pressure by modulating the activity of the autonomic nervous system. Modified baroreflex activity will however also change baroreflex gain and hence result in a complex feedback mechanism. An increase in baroreflex sensitivity means that smaller changes in blood pressure will give rise to alterations of autonomic nervous system activity. It is therefore impossible to predict the effect of medication on HRV using only a simple model. Furthermore, any effect, is likely to depend on the specific patient population that is studied. According to the neurohumoral concept, an overburdened heart loses its inotropic capacity, leading to lower cardiac output and less renal perfusion. This in turn results in activation of the renin-angiotensin system and of the baroreflex. A specific review article focusing on patients with left ventricular dysfunction and congestive heart failure was published by Tuininga et al[237]. These mechanisms lead to increased sympathetic activity, causing even more workload of the heart. In this paragraph, a simplified overview is presented of a number of cardiac drugs and their effect on HRV.

Placebo
In studies that compare active medication to placebo no effects of a placebo on HRV have been found. Two studies confirmed the absence of placebo effect. One study investigated normal subjects over a period of 3 - 65 days using time- as well as frequency-domain variables[121]. Another study also used time- as well as frequency-domain variables in a group of 40 patients after their first uncomplicated myocardial infarction[29]. Ambulatory monitoring immediately after infarction was compared to a recording three days after infarction. In this relatively short time span, no differences were found after the administration of placebo, while changes were observed after the administration of captopril.

Digoxin
In patients with congestive heart failure, digoxin has been shown to enhance baroreflex sensitivity and lower neurohumoral activation at low doses. Brouwer et al showed the effect of digoxin in patients with mild to moderate congestive heart failure, in a double-blind trial[38]. Patients were randomized to placebo, ibompamine or digoxin. After 3 months, pNN50, absolute and normalized high frequency power increased with digoxin, while this was not the case in the placebo group. These changes were observed during daytime hours only and were most pronounced in patients with the most impaired baseline HRV. Digoxin apparently enhanced cardiac vagal activity in the setting of neuroendocrine activation. These findings were confirmed by a study by Kruhm et al[127] that evaluated 4-8 weeks of oral digoxin therapy in congestive heart failure patients and found an increase in several HRV variables. They found that in normal subjects, digoxin also induced an increase of variables representing short-term fluctuations of RR-intervals without affecting the average RR-interval, however no influence on head-up tilt could be demonstrated[115].

β–blockers
The most obvious effect of a β–blocker is lowering of heart rate as a consequence of blocking sympathetic activation of the heart. Depending on the properties of the β–blocker, the effect of β–blockade is achieved by blocking receptors on the heart itself or by effects on the central nervous system. The decrease in heart rate will cause an increase in the variance of RR-intervals. As a consequence, virtually all HRV variables will increase, even LF, measured in absolute units. This is true for normal subjects[46] as well as post-myocardial infarction patients[209]. For normalized units LFnu may decrease. In patients after myocardial infarction, a significant difference in HRV variables was found after treatment with metoprolol using absolute (SDNN, pNN50) as well as relative variables[170]. β–blockers have also proven to be effective in the decrease of day-night patterning of HRV (circadian variation). An investigation assessing hydrophilic and lipophilic β–blockade revealed no differences[235]. In a double-

blind, crossover study, Brouwer et al[40] investigated the usefulness of heart rate variability in predicting drug efficacy (metoprolol vs. diltiazem) in 28 patients with stable angina, proven coronary artery disease, and myocardial ischaemia during Holter monitoring. Metoprolol significantly reduced the amount of ischaemia in patients with a low SDNN at baseline, while treatment effects were not observed in patients with a high SDNN at baseline. In contrast to metoprolol, the efficacy of diltiazem was not related to baseline heart rate variability. From this study, it was concluded that analysis of heart rate variability may be useful in selecting patients who will benefit from treatment with β–blockers.

In conclusion: When assessing the effect of β–blockade, one should be aware of the fact that a large part of the changes after β–blocker treatment are caused by a direct effect on heart rate. The effects of β–blocker treatment should be evaluated in both absolute as well as normalized variables of HRV. Preferably results should also be presented in CCV variables of HRV.

ACE-inhibitors

In patients with congestive heart failure, the long-term use of ACE-inhibition is associated with an increase of total as well high frequency components of HRV[26, 66]. Similar findings were obtained in a post myocardial infarction group[29]. In hypertensive patients, a clear decreasing effect of ACE-inhibition was shown using frequency-domain analysis (0.08 - 0.12 Hz band) in a group that was treated with 12 mg spirapril. A group that was treated with 3 mg spirapril showed no effect. Since no effects were present in the HF band, these authors concluded that this indicated an inhibitory effect of ACE-inhibition upon sympathetic vasomotor control and that the lack of difference in the HF band suggested that there was no change in the vagal cardiac drive. Obviously, the effect of ACE-inhibition on HRV may be related to the degree of neurohumoral activation[250]. Differences in working mechanisms between different ACE-inhibitors did not result in differences in HRV.

Calcium antagonists

To assess the effects of calcium antagonists on HRV, a distinction should be made between dihydropiridines and other calcium antagonists such as verapamil and diltiazem. The first group of drugs primarily causes vasodilatation, whereas verapamil and diltiazem also affect sinus rate and AV conduction. In 12 patients with proven coronary artery disease, diltiazem was administered for 2 weeks, after which an increase in the HF component (0.18-0.35 Hz) could be demonstrated during controlled respiration(0.25 Hz)[68]. In a group of post myocardial infarction patients diltiazem was shown to reduce LF while this effect was not observed in patients treated with nifedipine[14]. After the administration of isomazole, a phosphodiesterase-inhibitor with calcium-sensitising properties, Tuininga et al. [238] showed an increase of pNN50 and rMSSD in patients with moderate to severe CHF.

Anti-arrhythmic drugs
Decreased HRV post myocardial infarction has been shown to be a risk factor for cardiac death. Some anti-arrhythmic drugs may cause a decrease in HRV variables, such as sodium channel blockers which lower HRV because of their negative inotropic effect. This was shown in at least two studies[152, 268]. Moricizine, a class IC type agent, has been demonstrated to cause a decrease of SDNNindex and pNN50 in healthy subjects[225]. β–blocking effects of these drugs may also play a role, as was shown by Lombardi et al., who evaluated the effect of propafenone[139]. Without affecting average heart rate, propafenone caused a reduction of the low and increase of the high frequency component of HRV. Also an attenuated response to tilt table testing was demonstrated[139].

In the assessment of pharmacological therapy, its efficacy and dose related effects the analysis of HRV may prove to be useful. Also in the comparison of different drugs HRV analysis may play a role even more since effect of placebo of HRV are absent. The selection of patients who will benefit from therapy is also a possible application of 24-hour HRV analysis. The latter is especially convenient since in these category of patients ambulatory monitoring is already frequently applied to assess the status of the patient and the effects of medication.

2.5 HRV AND ATRIAL FIBRILLATION

HRV is normally calculated using sinus rhythm only, special care being taken to exclude NSI. However, HRV may also be of interest in non-sinus rhythm. In this respect atrial fibrillation in particular comes to mind. Atrial fibrillation is a common rhythm disturbance, especially in elderly people. During atrial fibrillation, the electrical behaviour of the atria is best characterized as "multi-wavelet re-entry" [7, 166-168]. This means that small wavelets all depolarize a part of the atria, constantly finding a new path of inducible tissue. From a clinical standpoint two forms of atrial fibrillation are distinguished: *paroxysmal* and *chronic* atrial fibrillation .

1. *Paroxysmal* atrial fibrillation (alternating relatively short episodes of atrial fibrillation and sinus rhythm). The autonomic nervous system is of importance in the genesis of this disorder. Changes in autonomic activity have been described as a cause of attacks[50, 206]. Characterisation of the cause may have profound influence on the treatment. Obviously beta blockers and digitalis will not be effective in preventing attacks in patients with vagally mediated attacks[50]. Therefore, it is of clinical importance to determine whether paroxysms of atrial fibrillation are sympathetically or vagally induced. Clinical history may provide an indication for the evoking mechanism. In addition, the behaviour of sinus rhythm preceding an

attack may be used to identify the underlying mechanism[50, 244]. From electrophysiologic studies it is known that stimulation of the vagus nerve results in shortening of the atrial refractory period and in an increase in the dispersion of refractory period in the atria[135]. This may well provide an explanation for the existence of so-called "vagal atrial fibrillation". It is a well known clinical fact that complaints vary strongly in seemingly equal attacks. HRV analysis may he helpful in determining the cause for complaints in patients with paroxysmal atrial fibrillation, since part of this effect may be due to baroreflex reactions to the cause and onset of atrial fibrillation. In chapter 9 an extensive case report of vagal atrial fibrillation is included.

2. *Chronic* atrial fibrillation is defined as atrial fibrillation lasting longer than 24 hours. The 24-hour cut-off point used for this definition is arbitrary, however it is based on a number of clinical facts and related studies. First of all, in clinical practice, atrial fibrillation lasting longer than 24 hours, almost never converts spontaneously to sinus rhythm. The duration of atrial fibrillation also has significant impact on thrombo-embolic complications risk. Whether the reverse holds true still needs investigation. Clinical experience indicates that atrial fibrillation >4 - 48 hours is associated with an increased risk for thrombo-embolic complications. Indeed, Weigner et al. showed a low likelihood of cardioversion-related clinical thromboembolism in patients with atrial fibrillation lasting less than 48 hours. Manning et al [158] et al. found left atrial thrombi in > 40% of patients with atrial fibrillation and acute thromboembolism. In another study, Manning et al. investigated whether the recovery of atrial systolic function was related to the duration of atrial fibrillation before cardioversion[157]. These investigators found that recovery of left atrial mechanical function was related to the duration of atrial fibrillation before cardioversion. They concluded that "these findings have important implications for assessing the early hemodynamic benefit of successful cardioversion". Also electrophysiological changes in atrial behaviour are noted within 24 hours as shown by Wijffels et al[259] and Tieleman et al[231] in chronically instrumented goats. They found that the median fibrillation interval shortened, the inducibility of atrial fibrillation increased and the atrial effective refractory period (AERP) shortened during the first 24 hours of atrial fibrillation. Crijns et al. showed that chemical cardioversion using flecainide is less successful when atrial fibrillation has lasted more than 24 hours[53]. In this form of atrial fibrillation, HRV may be used to study the autonomic modulation of atrial fibrillation. The autonomic nervous system is known to influence the electrophysiological properties of the intra-atrial signal during atrial fibrillation[84], as well as the

atrioventricular nodal conduction time. Electrophysiological studies have shown that the autonomic nervous system influences the electrophysiological properties of the atria[135]. Vagal activity shortens refractory period and leaves the conduction velocity unchanged. This causes a decrease in wavelength[218], therefore more wavelets fit into the atria, and the electrical signal of atrial fibrillation becomes fragmented. Since the effect of vagal stimulation is not equally distributed in the atria, the dispersion of refractory periods increase. Ventricular rate during atrial fibrillation is determined by the intrinsic atrial rate and the refractory period of the atrioventricular node[232]. The variations in ventricular rhythm are considered to be the consequence of the varying degree of penetration of the atrial impulses into the atrioventricular node, thus causing varying degrees of refractoriness ("concealed conduction")[73, 173] This means that HRV is - to a certain extent - also applicable during atrial fibrillation. In chapter 9 a study regarding autonomic influences during atrial fibrillation is presented.

Recently Hayano et al have published two studies that use HRV to characterize atrial fibrillation [87, 89] Describing spectral characteristics of atrial fibrillation, these authors confirm our finding that HRV during atrial fibrillation consists of an autonomically controlled irregularity and a basic irregularity inherent to atrial fibrillation. They conclude that the long-term fluctuations during sinus rhythm and atrial fibrillation may originate from similar dynamics of the cardiovascular regulatory system. In a study regarding AV-nodal properties the same authors found that refractory period as well as the degree of concealed conduction show a circadian rhythm. This finding may also constitute a basis for variations in ventricular rate during atrial fibrillation.

Future directions for HRV research in atrial fibrillation are ample. The various possibilities can be subdivided into analysis of sinus rhythm before or after and analysis of the ventricular signal during atrial fibrillation. Analysing sinus rhythm in patients with atrial fibrillation may be helpful in distinguishing between the various causes of atrial fibrillation such as sick sinus related or atrial premature beat related atrial fibrillation, or atrial fibrillation induced by changes in autonomic tone (vagally mediated or sympathetically mediated atrial fibrillation), thus setting the diagnostic stage for adequate treatment. The choice of treatment depends on the form of atrial fibrillation. Simultaneous registration and subsequent analysis of atrial and ventricular signals may further elucidate the behaviour of the AV-node during atrial fibrillation.

From a clinical standpoint characterisation of atrial fibrillation may be of value for:

1. determination of autonomic status during atrial fibrillation;
2. evaluation of drug treatment in for example anti-adrenergic, vagomimetic, anti-arrhythmic drugs,
3. the prediction of efficacy of cardioversion.

HRV analysis may provide insight in the evoking mechanisms of atrial fibrillation and present information about the status of the disorder. Furthermore, HRV may be used to evaluate the efficacy of for example vagomimetic drugs available for treatment and control of atrial fibrillation.

3
ECG REGISTRATION AND ANALYSIS

The essence of HRV analysis is mathematical computations on a series or RR-intervals. In order to obtain reliable results, a reliable input signal is essential. In this chapter an overview is presented of the different aspects of ambulatory monitoring that may influence the outcome of HRV analysis.

3.1 ECG REGISTRATION

3.1.1 Ambulatory monitoring — hook-up procedure

The hook-up procedure of ambulatory monitoring is the basis for ECG registration. A number of steps should be carried out to ensure a good quality ECG recording. The first step is skin preparation. This aims to remove the upper layer of the skin, ensuring low impedance and therefore a good electrical contact between skin and electrode. In order to assure a good quality registration rubbing the skin is often enough. The use of substances that leave a residue, such as stone powder, sandpaper or even alcohol may have a negative influence on the quality of the ECG.

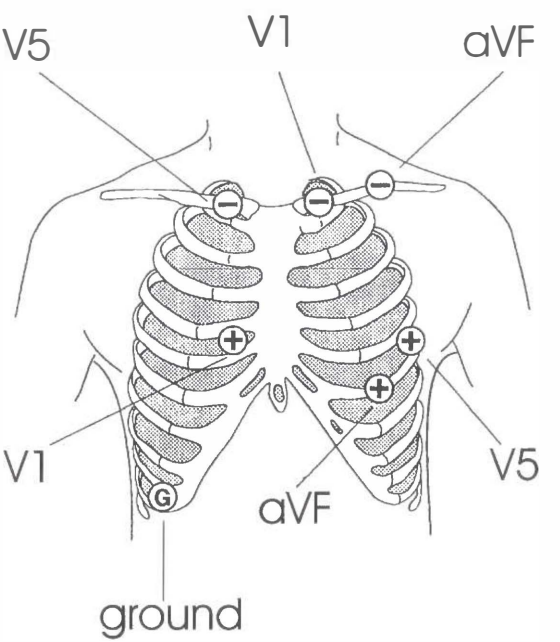


Figure 9. Holter hook-up scheme. Correct electrode position improves classification of the ECG and may therfore have a strong influence on the outcome of HRV calculations.

In ambulatory monitoring, modified leads V1 and V5 are usually used, sometimes in combination with a third channel such as aVF. The electrode placement for this specific hook-up scheme is shown in Figure 9. When selecting electrode position, regions covering muscles such as the pectoralis area should be avoided as much as possible, proper electrode placement will prevent excessive muscle artefact.

For ambulatory monitoring, ECG electrodes with special characteristics are needed:

- The adherence of the electrode must remain stable during the entire recording. This requires long-lasting electrode glue.
- The electrode that is used must "breathe" enough, in order not to loosen due to perspiration. This is especially important during summertime, when warm weather is an important cause of noisy ECG recordings.
- In order to prevent movement artefact, the material of which the electrode consists should be very thin and flexible in order to ensure that the electrode will follow movements of the skin.
- The electrode glue and gel should cause as little skin irritation as possible. Skin irritation causes patients to scratch, which is also an important source of noise.

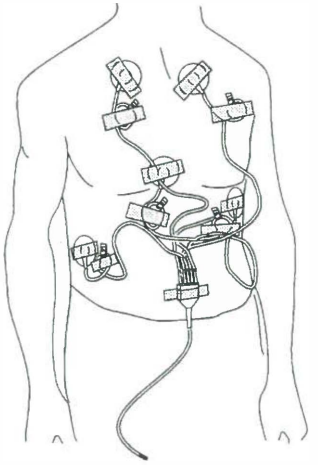


Figure 10. Stress loops. Using stress loops in the ambulatory monitoring hook-up procedure reduces strain of the lead-wires and thus decreases the amount of noise in the ECG recording.

When the electrodes are attached impedance measurement is the best test for proper electrode contact. Furthermore, an ECG test strip is useful to ensure proper hook-up. Finally, the lead-wires are attached to connect the electrodes with the Holter recorder. These lead-wires should be secured with tape in such a way that strain on the electrode is prevented (Figure 10).

3.1.2 AD conversion

After recording, the ECG signal is transferred to a computer system for analysis. During this phase the analog signal is digitized. When this process of digitising of the ECG is not carried out carefully, severe fluctuations in the reproduced ECG signal may occur. The most frequently used ambulatory monitoring equipment consists of an analog tape recorder, utilising two or three channels to record the ECG. The ECG must be reproduced perfectly, otherwise one would measure recorder variability instead of HRV. Possible tape speed irregularities are compensated for by means of a time track that is recorded on one of the tracks of the tape (mostly 32 Hz block pulse). This signal regulates the tape playback speed and the timing of the AD-converter in order to reproduce the ECG accurately (phase-locked loop circuit). Ambulatory monitoring tape recorders without a phase-locked loop circuit are not suitable for HRV analysis[2]. Most recorders use an AD-sampling frequency of 128 Hz, resulting in a sampling distance of 1000/128 ≈ 8 ms. The influence of this sampling frequency on HRV computations has been investigated in several studies[3, 163, 252]. A measurement error of approximately 2% may occur in healthy persons, whereas in patients with low HRV the error may amount to as much as 8.3% . A sampling frequency below 100 Hz is generally considered inadequate for HRV analysis[2, 252], while sampling frequencies of 256 Hz and higher result in an almost error free operation in most cases[252]. In patients with extremely low HRV (e.g. cardiac transplant patients) an error of up to 100% may occur[3]. Therefore, HRV changes in patients with low variability must be considered extra carefully when recorded using standard 128 Hz commercially available ambulatory monitoring systems.

3.2 ECG ANALYSIS

Once the ECG is recorded, QRS complexes must be detected and classified: the ECG analysis. This process of ECG analysis is performed by computers and, when necessary, corrected manually. Since the entire HRV computation is based on RR-interval series, this process is of great importance. A description of this process, its pitfalls and some focus points are described in the paragraphs 3.2.1 and 3.2.2.

3.2.1 QRS detection - detection and classification of QRS complexes

After AD conversion, a computer algorithm scans the signal for peaks that could be QRS complexes. After all peaks have been identified, peaks that probably are not QRS complexes - e.g. muscle artefact - are eliminated by complex computations based on slope, amplitude and other properties of a peak. Three possible problems can occur in the detection/classification of QRS complexes:

1. Failure to detect beats; beats are not detected, for example due to noise or small amplitude.
2. Overdetection of beats; a "non-existent beat" is detected, for example due to a pointed T-wave.
3. Misclassification; the beat is correctly detected, however it is considered normal while it is ectopic or vice versa.

If proper manual overreading is performed, these problems can be corrected and will have little influence on the outcome of HRV analysis (see chapter 3.2.3). In most ambulatory monitoring analysis systems, the result of the computerized ECG analysis is a group of templates, each of which consists of beats with a high level of similarity. Also graphs such as histograms and trends are created to allow the analyst to verify items like the length of RR-intervals, prevalence of ectopics and levels of ST-segments.

3.2.2 QRS detection - influence of QRS width

The detection and to some extent the classification of QRS complexes in ambulatory monitoring are performed by a computer algorithm. Detection of beats is performed using amplitude and/or first derivative of the QRS complex as parameters. After beat detection. groups of look-alike QRS complexes are created that are represented by a template. In these templates information about the morphology of the P-wave is virtually absent. Therefore, in ambulatory monitoring it is common to differentiate between beats of sinus origin and beats of supraventricular ectopic origin by means of prematurity. The coupling interval of normal QRS complexes is compared to the preceding interval and when it exceeds a certain (operator-controlled) limit, a beat is considered of supraventricular ectopic origin. The onset of QRS templates is usually not changed by the editor. However, inconsistent determination of QRS onset may result in artificial variations, especially in ECGs with wide QRS complexes (> = 120 ms), since wide QRS complexes have a more gradual slope, and the onset of wide QRS complexes may therefore be more difficult to determine than the onset of narrow QRS complexes (< 120 ms) which have a relatively steep slope. In order to study these effects, we performed a study in healthy volunteers and congestive heart failure patients[81].

The study was performed in 10 apparently healthy subjects (mean age 43 ± 10) and 9 congestive heart failure patients (mean age 64 ± 11). The healthy subjects had no history of major disease. Physical examination and ambulatory monitoring revealed no abnormalities. During the actual protocol, the ECG was recorded using a four-channel Marquette series 8500 recorder. This recorder utilizes a 32 Hz time track to compensate possible tape speed irregularities. The analysis was performed using a Marquette 8000 XP analysis system (PDP 11-23, using a sampling frequency of 128 Hz.). This system is connected to a NFS-based PC network using an IBM RS6000 as a server for data storage and retrieval. The ECG was visually checked for ectopic beats and noise by an experienced analyst. After the ECG analysis the RR-interval series was transferred to the COHORT system for detailed HRV analysis. The COHORT (COmputerized HOlter Research Tool) system is a locally developed software package (written in DELPHI™ and FOXPRO®) used as a post processor for detailed HRV analysis. This RR-interval series therefore was based on automatically triggered QRS templates. Next, the onset of each template was corrected manually using an especially developed tool (CCTOC). This tool allowed the operator to review magnified templates and correct onset points when necessary. This resulted in a RR-interval series based on manually triggered QRS templates. Time-domain analysis was performed over the full 24 hours, while frequency-domain analysis was performed over 5-minute segments using discrete Fourier transformation [2, 58, 83, 203]. Only data segments containing less than 5% noise or ectopy were accepted for analysis. This is a percentage based on time, not number of beats [83]. Finally a check was performed to ensure the validity of the frequency-domain analysis by comparing the square root of the overall spectral power to SDNN ((sqrt(total frequency power) / SDNN)*100%). Parseval's theorem(Equation 1) states that the power in time domain is equal to the power in frequency domain. Therefore, the result of this computation (Parsevals index) should be 100%. Deviations of the Parseval index can be caused by non-stationarity. All deviations >10% were excluded from further analysis. All HRV variables were computed in accordance with the guidelines as written by the HRV task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology[2]. For statistical analysis SPSS version 7.5 was used. Students-*t* test was used to analyse the data.

It was expected that the result of the arrhythmia analysis would not differ, since the only modification that was performed was the adjustment of the QRS onset point. However in one patient this QRS onset adjustment caused such a difference in QRS timing that the number of supraventricular ectopic complexes changed from 263 to 1804. The consequence of this is that more episodes were excluded due to the prevalence of NSI.

Variable	QRS < 120 ms		QRS >= 120 ms		difference (%)
	auto	manual	auto	manual	
AVGNN	804	804	810	811	0
SDNN	145	145	168	168	0
SDANN	160	160	184	186	0
SDNNindex	55	55	60	60	0
rMSSD	31	31	38	38	0
TP	3706	3720	4224	4311	-3
VLF	2447	2460	2876	2915	-3
LF	874	876	963	974	-2
HF	385	384	385	422	-9
LFHF	3.8	3.8	4.4	4.0	-10

Table 4. HRV variables averaged over 24 hours, before and after manual correction of QRS onset in patients with narrow and wide QRS complexes.

Variables averaged over 24 hours.

The result of time-domain analysis over the full 24 hours and frequency-domain analysis averaged to 24-hour means are shown in Table 4. For the frequency domain variables this means that 5-minute results are averaged to a 24-hour mean. These mean values are in turn averaged to a group value. The results show that the effect of manual QRS onset correction leads to almost no differences in patients with narrow QRS complexes, while in subjects with wide QRS complexes a decrease up to 9% (LFHF) and an increase up to 10% (HF) can be seen.

A comparison of congestive heart failure patients and normal subjects is shown in Table 5. In both groups differences occurred as a result of manual correction. For instance in congestive heart failure patients HF increased 9% while in the normal subjects LFHF decreased 9%.

Variable	Congestive heart failure patients			Controls		
	auto	manual	difference (%)	auto	manual	difference (%)
AVGNN	836	837	-	781	781	-
SDNN	154	155	-	155	155	-
SDANN	165	168	-2	174	174	-
SDNNindex	55	55	-	59	59	-
rMSSD	34	34	-	33	35	-6
TP	3491	3535	-2	4314	4360	-2
VLF	2680	2697	-1	2580	2611	-2
LF	565	570	-1	1223	1230	-1
HF	245	268	-9	511	519	-2
	LFHF	3.7	3.6	+2	4.5	4.1 +9

Table 5. HRV variables averaged over 24 hours, before(auto) and after manual correction of QRS onset in normal subjects and patients with congestive heart failure.

	QRS < 120 ms		QRS >= 120 ms	
	delta	95% CI	delta	95% CI
AVGNN	0.012	0.01 - 0.02	0.05	0.01 - 0.09
HF	6	6 - 8	25	21 - 30
LF	5	4 - 6	15	8 - 22
TP	9	8 - 10	49	24 - 74
LFHF	0.13	0.11 - 0.15	0.6	0.6 - 0.7
HFnu	0.5	0.5 - 0.6	1.5	1.3 - 1.7
LFnu	0.6	0.5 - 0.6	1.5	1.3 - 1.7
ccvLF	0.01	0.00 - 0.01	0.02	0.02 - 0.03
ccvHF	0.03	0.02 - 0.03	0.07	0.06 - 0.08

Table 6. Absolute difference (delta) as consequence of manual QRS onset correction in narrow and wide QRS complexes.

Variables computed per 5 minutes.

Besides group averages over 24 hours, also an analysis on a per 5-minute basis is of interest since sequential short-term analysis is often used to study transient events such as ischaemia. The absolute differences (delta) and confidence intervals of HRV variables computed over 5 minutes that occurred as a consequence of manual QRS onset correction were averaged. These averages with the 95 % confidence intervals are shown in Table 6.

All changes in Table 6 proved to be highly significant. The effect of manual correction was more pronounced in the wide QRS complex group than in patients with narrow QRS complexes. In order to demonstrate know to what extent differences may occur within 5-minute segments, the values of the segment with the maximum change is shown in Table 7, together with the accompanying ratio (before correction/after correction).

	QRS < 120			QRS >= 120		
	A	M	ratio	A	M	ratio
AVGNN	**886**	**887**	1.0	**1145**	**1181**	1.0
TP	**728**	**928**	1.3	**32070**	**10494**	.33
LF	**70**	**133**	1.9	**767**	**204**	.27
HF	**13.3**	**49.8**	3.7	**14.8**	**94.5**	6.4
LFHF	**10.2**	**2.8**	0.3	**12.5**	**1.9**	.15
LFnu	**78**	**56.0**	0.7	**69.7**	**23.7**	.47
HFnu	**9.0**	**27.0**	3.0	**7.4**	**34.1**	4.6
ccvLF	**1.3**	**1.7**	1.3	**2.3**	**1.7**	.53
ccvHF	**2.8**	**1.5**	0.5	**0.6**	**1.4**	2.3

Table 7. HRV variables before (A) and after (M) manual correction and accompanying ratio computed over 5-minute segments in recordings with narrow (QRS < 120) and wide (QRS >=120) QRS complexes.

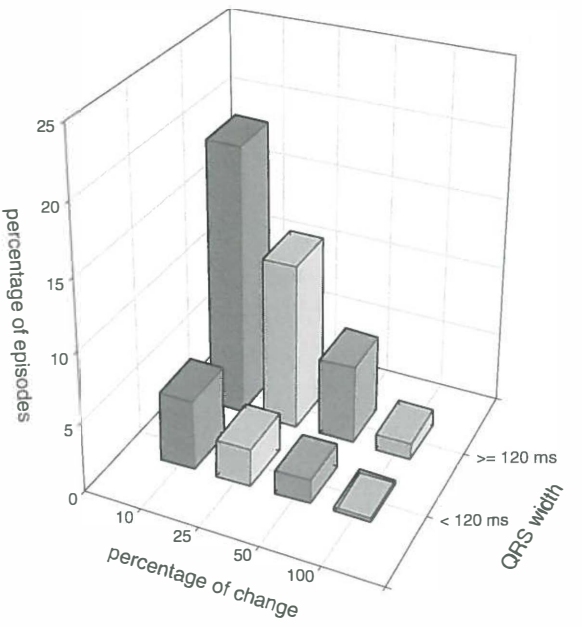


Figure 11. Percentage of 5-minute segments where rMSSD exceeded the predefined cut-off limits in wide and narrow QRS complexes, as a result of manual correction of QRS onset. In subjects with wide QRS complexes, correction of QRS onset leads more often to differences in rMSSD compared to subjects with narrow QRS complexes.

We also investigated how often changes exceeded 4 limits: 10, 25, 50 and 100% (Results are given in Figure 11 and Table 7).

It is obvious that on average changes as a result of manual correction occurred more frequently in subjects with wide QRS complexes than in subjects with narrow QRS complexes.

	10%		25%		50%	
	N	W	N	W	N	W
AVGNN	0.0	0.0	0.0	0.0	0.0	0.0
TP	0.2	2.0	0.1	0.2	0.0	0.0
LF	0.3	1.8	0.1	0.4	0.0	0.1
HF	5.0	21.2	2.5	13.1	1.4	9.0
LFHF	4.6	2.2	1.1	0.7	0.1	0.2
LFnu	0.3	0.4	0.0	0.1	0.0	0.0
HFnu	4.2	17.9	2.1	11.5	1	6.9
ccvLF	0.2	0.6	0.0	0.1	0.0	0.0
ccvHF	3.4	15.6	1.3	7.3	0.4	0.2

Table 8. Percentage of episodes where HRV variables exceeded the predefined cut-off limits in wide (W) and narrow (N) QRS complexes.

Accurate detection of QRS onset plays an important role in the outcome of HRV analysis. In patients with wide QRS complexes, changes due to differences in QRS onset detection occur more frequently and are more prominent than in patients with narrow QRS complexes. Wide QRS complexes have a more slurring onset; a consistent trigger point is therefore harder to establish. Furthermore, we observed that ECGs with wide QRS complexes vary more in morphology, a factor that also contributes to the inconsistent triggering. Average 24-hour results of HRV analysis are influenced to a lesser extent than single 5-minute computations. Average 24-hour results may deviate up to 10% in subjects with wide QRS complexes, while in subjects with narrow QRS complexes, virtually no effect of manual QRS onset correction is seen. Also HRV variables that measure beat-to-beat changes are more sensitive to changes in QRS onset than variables that measure slow fluctuations in RR-intervals. Because of a possible effect on the outcome of supraventricular ectopic counts, the adjustment of SVE prematurity in ambulatory monitoring recordings should be performed after the QRS onset correction.

Limitations: *Although the templating method is used in virtually all Holter analysers, QRS detection algorithms are different in commercially available systems. Therefore, the results of this study apply to the Marquette XP Holter analyser using software revision 5.8. In other Holter-analysers the impact of the phenomenon may vary.*

3.2.3 QRS classification — influence of ectopic beats

Cause and amount of non-sinus intervals

In order to perform reliable HRV analysis, proper identification and removal of NSI intervals is needed. NSI consist of noise and ectopic beats. It is important to realize that NSI may be reported as a percentage of time or as a percentage of intervals and that these percentages may differ. The lack of relation between the percentage of ectopic beats in a recording and the percentage of NSI, measured in time, is shown in Figure 12, based on recordings obtained from 419 healthy subjects (for a more detailed description of these subjects see chapter 5).

A substantial percentage of NSI in time may exist, although the percentage of NSI expressed as a percentage of beats is low. Therefore, in a study the percentage of NSI should also be reported as a percentage of time, not only as a percentage of beats. Less ectopy obviously leads to better results of HRV analysis. Although the influence of ectopic beats has been the subject of a number of studies[28, 42, 253], no recommendations are presented with regard to the maximum percentage of NSI acceptable for HRV analysis. Different investigators use different NSI limits, varying from 5%[80] to 40%[119], expressed as a percentage of beats. Besides the unpredictable effect of ectopic beats on

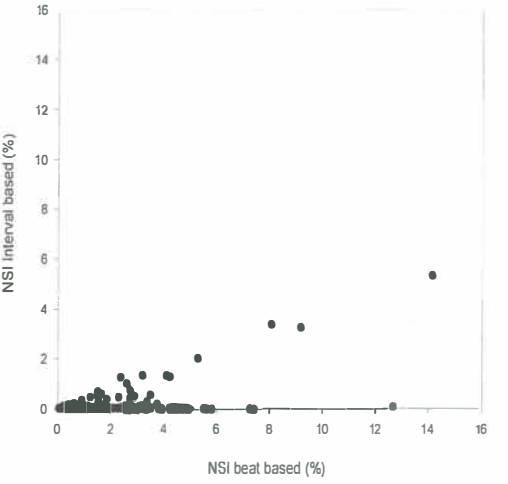


Figure 12. Relationship between time- and interval-based non sinus intervals (NSI) in 419 healthy subjects. The percentage of NSI expressed as a percentage of beats is consistently lower than the percentage of NSI expressed as a percentage of time.

the sinus node, the difficulty in determining a cut-off point also lies in the fact that excluding segments with too much ectopy leads to less data. In individuals with a large amount of ectopy this may lead to a situation where the remaining data no longer represent the 24-hour average. Ectopic activity as a consequence of altered autonomic output, is often not equally distributed over the 24 hours. In addition, exclusion may therefore lead to a selection bias: the remaining data does not represent the original signal. Although in out opinion limits with respect to the amount of NSI that may be accepted for analysis cannot be given, data segments containing more than 15% of time-based NSI, should always be excluded from analysis. Data segments containing less than 5% of time-based NSI, may be safely used for the analysis of HRV.

Identification and influence of ectopic beats

Since the autonomic nervous system mainly exerts its influence through the sinus node, sinus intervals are the only type of intervals that should be included in the analysis of HRV. Unfortunately, this is not always possible. In particular, virtually endless numbers of different QRS morphologies may be found in patients with frequent multiform ventricular ectopic beats. Especially in a cardiologic patient population, rhythm disturbances may occur frequently. Ambulatory monitoring analysis equipment is designed to detect and classify NSI, and allows the analyst to verify beat classifications and correct these whenever necessary. Ectopic beats may be divided into 2 types: supraventricular and ventricular. In HRV analysis, simply excluding these beats is not a perfect solution, because both types of ectopic beats may cause a reset of the atria including the sinus node, thus disturbing the "natural" sinus rhythm. Supraventricular ectopic beats virtually always cause a reset of the sinus node,

while ectopic beats originating from the ventricles may result in retrograde conduction over the atrioventricular-node, thus also leading to a reset of the atria and sinus node in approximately 50% of all cases. Furthermore, the occurrence of ectopic beats may cause symptoms, which, in turn may lead to autonomic activation due to fear or anxiety, thus influencing HRV. Moreover, ectopic beats result in a varying cardiac output which may also, via the baroreceptor feedback loop, influence the trailing sinus rhythm. Some investigators have suggested to exclude even more than one interval before and after ectopic beats. This however, is not approved of by others[42]. In this study the loss of extra data did not result in a better outcome of HRV analysis. One study in congestive heart failure patients investigated the difference of 10 NN-intervals before and after a premature ventricular contraction (PVC)[263]. The investigators found no differences in the behaviour of NN-intervals before and after the PVC and concluded that the exclusion of extra intervals around an ectopic beat will not result in an improved outcome of HRV analysis.

Automatic exclusion versus manual identification of ectopic beats

The ultimate consequence of the above described phenomena is, that the "natural interval sequence" of the sinus rhythm, i.e. the rhythm that would have occurred if the ectopic beat would not have interrupted the natural sequence, cannot be predicted. Automatic correction of ectopic beats is therefore impossible. Even though the guidelines state clearly that: 'Automatic "filters" that exclude some intervals from the original RR sequence should not replace manual editing', complete or partial automatic detection of NSI is frequently applied in studies[104, 122, 123, 169, 194]. Usually this is advocated by the fact that "manual review" of ambulatory monitoring is time consuming, requires experienced personnel and hence, is a costly procedure. Some investigators have tried to develop improved algorithms for automatic exclusion of NSI[131, 132]. In most studies, the use of such an algorithm, a so-called percentile exclusion rule, is reported, however, the exact methodology is seldom explained. Malik et al. have investigated the use of a number of automatic filters for ectopy exclusion and concluded that all of these fail to detect ectopic beats adequately[147]. These investigators propose the use of more robust variables of HRV(TINN and Triangular index, see chapter 4.1.3). Whatever method is used to detect and/or exclude ectopic beats, the maximum percentage of accepted ectopic beats in a study should always be stated. Moreover, this percentage should be reported, not only as a percentage of beats but also as a percentage of time. Since every ectopic beat has a coupling interval and a compensatory pause, the percentage of time excluded due to ectopics, roughly doubles the percentage of beats excluded. All intervals that are not preceded and followed by a sinus beat should be considered NSI and therefore these intervals must be excluded from HRV analysis. Ectopic beats can occur prematurely as well as late (escape beats) or sometimes in the same frequency as the prevailing sinus rhythm.

Obviously, the latter are the most difficult to distinguish from sinus rhythm if the detection is only based on interval criteria as opposed to morphological criteria. The most frequently used percentile exclusion rule is the 20% exclusion rule. This technique classifies an interval as NSI if the RR-interval deviates 20% or more when compared to the preceding interval. As an example, an ECG with a PVC is shown in Figure 13. A 20% exclusion rule would define the second interval (I2) and the third interval (I3) as a NSI because I2 exceeds 20% prematurity when compared to I1 and interval I3 because it is more than 20% longer than I2. When using morphological criteria, I2 and I3 would be excluded also because of the PVC. Interval 4 (I4) is considered a NSI according to the percentile exclusion rule (over 20% premature when compared to I3), but not by morphological criteria. A solution often used for this problem is holding I1 as the last "normal" interval until another interval within the 20% range is reached.

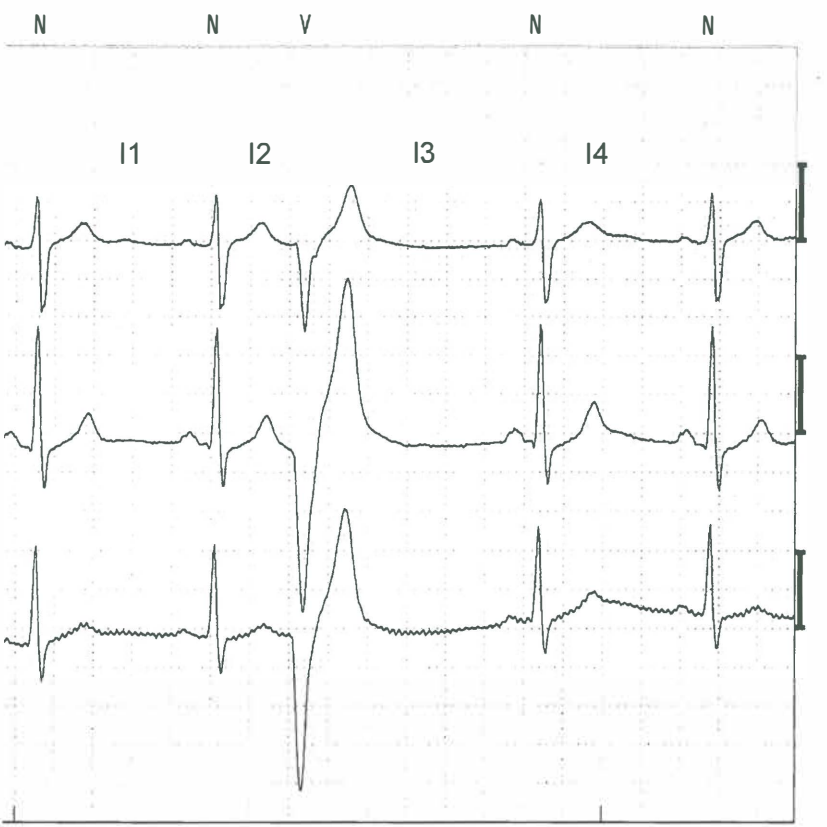


Figure 13. Premature ventricular contraction. A 20% exclusion rule would define interval 2 (I2) and interval 3 (I3) as NSI because I2 is >20% premature when compared to I1 and interval I3 is >20% longer than I2.

However, in case of a sudden increase or decrease in heart rate, this solution may lead to very long periods of "false positive" NSI detection, hence resulting in incorrect exclusion of normal intervals. Furthermore, in a number of conditions automatic exclusion of NSI based on prematurity cannot work properly:

I. In healthy, especially younger individuals, e.g. a control group in a study, most intervals that exceed 20% difference compared to the preceding interval are the consequence of "normal" sinus irregularity. Furthermore, true ectopic beats are relatively infrequent in this group. Therefore, the use of a percentile exclusion rule will exclude mainly true sinus intervals and thereby cause an underestimation of true variability.
II. In a population with decreased variability (e.g. congestive heart failure patients) ectopy occurs more frequently and true variability is less marked. Also longer repetitive sequences may occur (tachycardias and idiopathic rhythms) which cause inadequate detection of ectopics by a percentile exclusion rule.
III. Not all rhythm disturbances differ from the normal rhythm with respect to timing, such as (supra)ventricular rhythms.
IV. The degree of prematurity of ectopic beats may vary between patient populations.

In order to further address the effect on automatic exclusion of NSI by means of a percentile exclusion rule we compared the use of such a rule to the "gold standard": manual review of the data.
Three patient categories were studied:

I. normal subjects (individuals without any apparent disease and without complaints),
II. patients with proven coronary artery disease defined as previous infarction or abnormal angiogram or positive thallium test combined with a positive exercise test.) ,
III. patients with congestive heart failure (New York Heart Association class III or IV).

All three groups consisted of 20 patients, predominantly male, with an average age of 59 years. Furthermore four patients from the three categories with different percentages of ectopy were selected and analysed separately to study the effect of the amount of NSI. The average duration of a recording was 23 hours en 42 minutes; no recordings less than 16 hours in duration were used. For the purpose of this study only subjects with >24 ectopic beats (avg. >1 per hour) of each type (ventricular and supraventricular) were accepted. Table 9 shows the average coupling interval (CI) and average prematurity of supra-ventricular as well as ventricular ectopy. It can be concluded that absolute timing (CI), relative timing (prem) and prevalence of ectopic beats strongly differ. The ability of a percentile exclusion rule to detect NSI is shown in Figure 14

	Ventricular		Supra-ventricular		Noise	Ventricular	Supra-ventricular	Total
Group	CI (ms)	prem (%)	CI (ms)	prem (%)	(min)	(min)	(min)	(min)
normal subjects	495	40	602	31	19	8	7	34
coronary artery disease	499	39	601	34	28	35	7	70
congestive heart failure	581	26	578	28	20	34	7	61

Table 9. Characteristics and prevalence of ectopic beats and noise. CI = couplings interval (ms), prem = prematurity (%)). The last three columns show the composition of NSI (number of minutes in a 24-hour ambulatory monitoring recording).

using the same patient groups. Percentile exclusion rules of 10, 20 and 30% were compared to manual classification of the ECG by comparing to total amount of NSI detected.

Figure 14 indicates that detection of NSI by the 30% exclusion rule resembles most closely manual detection. Setting the percentile exclusion rule to 10% led to approximately a 280% increase of the NSI duration in normal subjects, while the best performance of the overall best setting (30%) still deviated 12% (61 vs. 68 min. in Group III). The frequently used 20% percentile exclusion

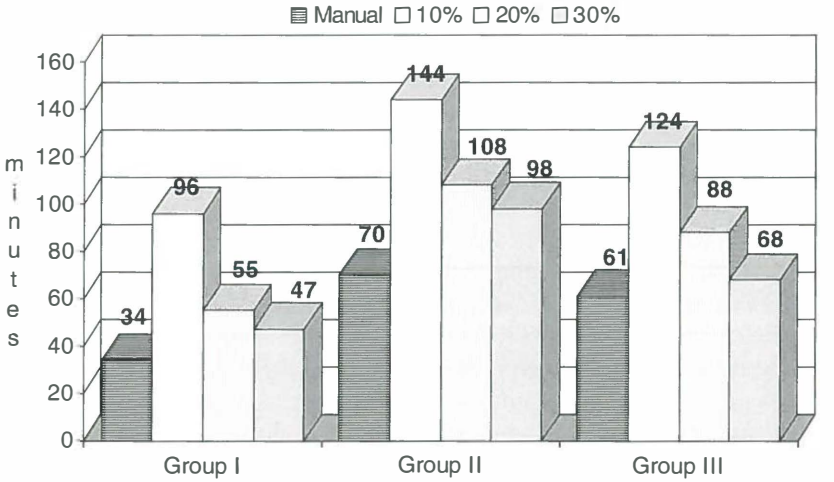


Figure 14. Duration of NSI by manual analysis and by use of a percentile exclusion rule in normals (group I), coronary artery disease (group II) and congestive heart failure (group III). The 30% percentile exclusion rule resembles manual detection the most.

Group/percentile exclusion rule	10%	20%	30%
I	0.5	0.7	1.9
II	0.3	1.1	3.5
III	5.4	11	16.7

Table 10. Duration of false negative detections (minutes)

rule led to a false positive exclusion of 21 minutes in normal subjects, 38 minutes in Group II and 27 minutes in Group III. Another complicating factor in using a percentile exclusion rule can be the occurrence of false negative detections, i.e. ectopic beats that are not classified as such by the percentile exclusion rule (Table 10).

The false negative detections prove to be of considerable duration, especially in group III. This may be explained by more frequent occurrence of couplets and (regular) ventricular tachycardia in this patient category. The application of a percentile exclusion rule did not influence the average sinus interval (heart rate) and the effect on other variables varied strongly.

Influence of ectopic beats on time-domain HRV

In theory, if the analysis of the ECG is performed well, the prevalence NSI is not a troublesome factor in the computation of time-domain HRV, since NSI intervals are simply skipped in the computation.

Figure 15 shows the effect of a percentile exclusion rule on the outcome of pNN50, in the 3 previously defined patient groups (I: Normals, II: coronary artery disease patients and III: congestive heart failure patients). The x-axis label "Real" represents manual detection of ectopic data. From this figure it can be concluded that the 30% exclusion rule approximates manual detection more closely rather than the more frequently used 20% exclusion rule.

When adequate analysis of the ECG cannot be performed, the effect of NSI depends on type (noise or ectopy) and amount of NSI. Detection errors and misclassifications will have a substantial impact on the outcome of time-domain analysis. A good example of the consequence of misdetection is shown in the PhD thesis of Mulder[178], who demonstrated that failure to detect 1 QRS complex leads to just as much variance as the total variance of a series of 100 intervals with a variation coefficient of 10% ($SDNN^2$). The importance of ECG analysis with respect to time-domain variables is shown by Malik[142]. This study compared manual editing of ECG with no editing. He showed that the different time-domain variables vary in sensitivity to misclassification of QRS complexes. Especially sensitive were SDNN and SDANN, demonstrating up to 500% deviation in patients with low HRV. In a number of patient subgroups

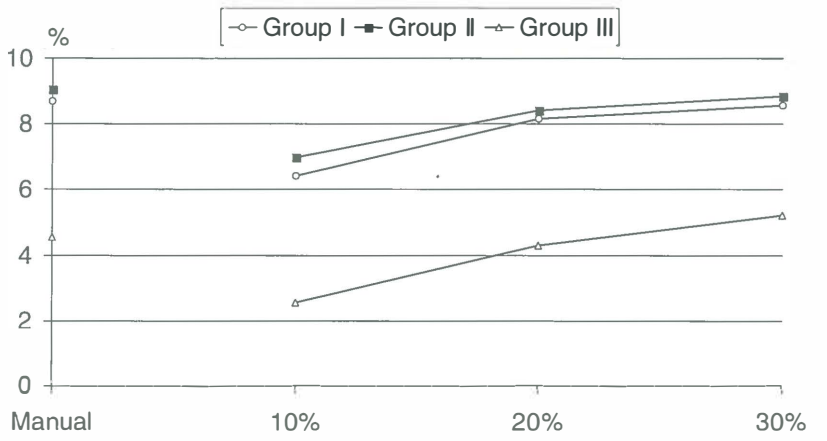


Figure 15. The effect of a percentile exclusion rule on pNN50 in three groups: Normals (I), ischaemic heart disease (II) and congestive heart failure (III). X-axis: method of detection, Y Axis: percentage of NSI detected. The 30% percentile exclusion rule resembles manual detection the most.

a low HRV is accompanied by an increased number of ectopic beats, thus increasing effect of HRV deviations due to QRS misclassification. Unfortunately the study by Malik did not address the influence of the amount of NSI on the outcome of HRV. As explained in chapter 2.4.2 the perfect solution for correction of NSI does not exist. Automatic exclusion of ectopic beats is not feasible either.

Influence of ectopic beats on frequency domain

While in time-domain variables NSI episodes are simply discarded, in frequency-domain analysis a continuous signal is required. Therefore replacement of NSI is performed. Figure 16 shows an example of this so-called substitution. The upper trace (A) shows an ECG with a PVC before substitution, while in the lower trace (B) this PVC is removed and replaced by an imaginary beat thus creating a N-n-N sequence in stead of a N-V-N sequence. In this case linear interpolation is used as a substitution method. There are two important aspects in the interpolation of ECG:

1. The timing of the beat after an episode NSI should not be altered, in other words long time fluctuations should not be influenced. The consequence of incorrect substitution is shown in Figure 17. The upper panel shows an artificial signal with a sinusoidal modulation, the original signal. The arrow denotes a segment where ectopic data are assumed. When this segment is simply removed, the result is as shown in the lower panel. In the lower panel three important characteristics of such imprudent removal can be seen:

Figure 16. Interpolation of noise. The fourth beat of the upper tracing (A) is a PVC, which should be excluded from HRV analysis. For the purpose of frequency-domain analysis the coupling interval (420 ms) and the compensatory pause (1100 ms) are added up and divided in equal parts, resulting in two intervals of 810 ms, as shown in the lower tracing (B).

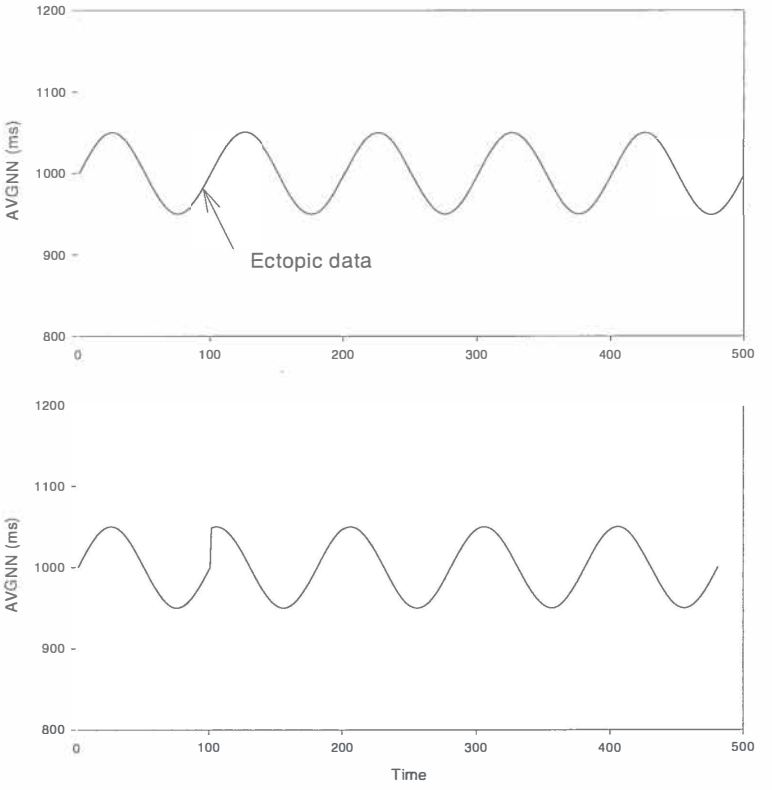


Figure 17. Incorrect substitution. The mere extraction of data without leaving successive beats in place leads to extreme frequency jumps and an incomplete data segment (the lower tracing is shorter).

- Sharp angles (high frequency) occur at the point where the data are removed and where the original signal is "reconnected".
- A large segment of time is not accounted for; the total amount of signal does not add up to the original 500 seconds.
- The timing of all beats after the removed segment is altered, this leads to different low frequency contents of the signal.

2. The total amount of time and beats substituted should not exceed certain limits. There is no consensus in the literature about the maximum amount of NSI that is acceptable (see chapter 3.2.3), however accepting only episodes with less than 5% NSI is generally considered safe. Automatic exclusion of ectopic beats will influence outcome of frequency-domain analysis strongly.

Effects of a percentile exclusion rule on frequency-domain HRV computations
Figure 18 shows the effect of 3 percentile exclusion rules on HF power in the 3 groups (I: Normals, II: coronary artery disease patients and III: congestive heart failure patients). The second group shows a strong deviation, not only from the actual value, but also relative to the other groups. This result in group II is mainly caused by 1 patient with a high percentage of ectopy. Note that for these computations all episodes with > 5% substitution are excluded from the analysis.

To investigate the influence of the amount of NSI, we selected four patients per category with different percentages of ectopy (± 1,3,5 and 20% ectopic beats on the ambulatory monitoring recording). Results are shown in Figure 19. As mentioned before, the amount of beats only reflects approximately 50% of the time of NSI. The legend shows the total duration of NSI as a percentage

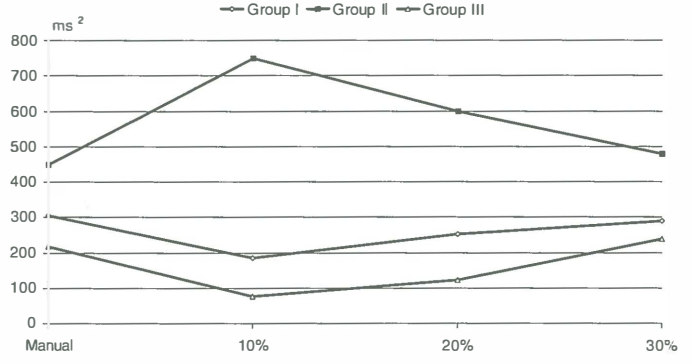


Figure 18. Effects of a percentile exclusion rule on high frequency power (0.15 - 0.40 Hz) in the 3 groups: Normals (I), ischaemic heart disease (II) and congestive heart failure (III). The second group shows a strong deviation, not only from the actual value, but also relative to the other groups. This means that a percentile exclusion rule leads to unpredictable behaviour and cannot simply be compensated for.

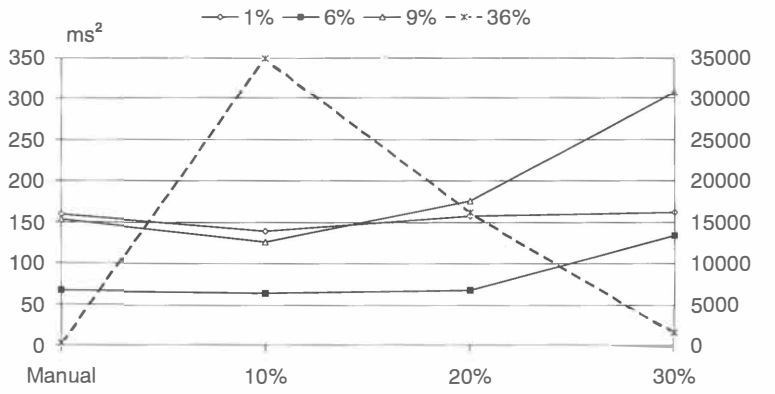


Figure 19. Effects of prevalence of NSI on high frequency power (0.15 - 0.40 Hz) . Four groups with 1, 6, 9 and 36% time-based NSI are shown. In the group with most frequent NSI, the 10% exclusion rule shows a strong deviation, while the 20 and 30% exclusion rule perform better, but still show a substantial overestimate of HF power. The solid lines refer to the left Y-axis, while the dotted line refers to the right Y-axis.

of time. The lines representing 1, 6 and 9 percent NSI show clearly that when the amount of NSI increases, the percentile exclusion rule starts to deviate more. The percentile exclusion rule values of the dotted line (36% NSI) deviated to extreme values: 34886, 16189 and 1489 ms^2 for the 10, 20 and 30% exclusion rules respectively. When manual NSI detection was used, 2 patients of the highest category had no data segments with < 5% NSI and could therefore not be included in the analysis. When using a percentile exclusion rule datasegments of these patients did contribute due to the false negative detections. Due to these false negative detections highly irregular signal was included in the analysis, leading to strong deviations in the outcome of frequency-domain analysis. It is concluded that the use of a percentile exclusion rule is insufficient to detect NSI adequately. The effect of a percentile exclusion rule on the outcome of HRV analysis depends on the amount of ectopy, the way this is dealt with and the HRV analysis method that is used.

In conclusion: manual editing of the ECG is mandatory. Adding a percentile exclusion rule to manual editing has no rational grounds when manual editing is performed accurately. Using a percentile exclusion rule without manual editing leads to unpredictable results of HRV analysis. Since there is no possibility to determine how the sinus node would have behaved if ectopy would have occurred, an exact cut-off point for the amount of ectopy that can be accepted for HRV analysis cannot be computed. If recommendations to a cut-off point can be made, these will at least be limited to the population in which it is assessed and is not necessarily valid in other patient groups. Also, it should be noted that excluding episodes with relative high prevalence of NSI may introduce a selection bias[131, 132] since increased prevalence of ectopy may cause changes in autonomic nervous system activity and vice versa.

4.
HRV ANALYSIS METHODS

This chapter presents an overview of the different techniques used nowadays. Although the guidelines suggest otherwise, there is no consensus about the methodology, especially with respect to frequency-domain analysis. There is no such thing as "the best technique", however there are a number of obligatory steps for reliable HRV analysis. The choice of a technique should depend on the underlying physiological model that is assumed.

From a mathematical point of view, one can distinguish linear and non-linear methods of HRV analysis. Linearity and non-linearity are concepts that originate from mathematics. In linear systems a + b = b + a and a * bc = bca, while in non linear systems this is not true. In HRV, linear methods focus on relatively simple mechanisms such as repeating or rhythmic changes in the successive RR-intervals, like the ± 0.25 Hz oscillation due to respiration. In non-linear analysis, less obvious, more complex relations are examined. In this chapter, the following methods to analyse HRV will be discussed:

1) Linear methods
   - time-domain analysis
   - frequency-domain analysis
   - geometrical analysis

2) Non-linear methods
   - chaos
   - fractal dimension
   - approximated entropy

4.1 LINEAR ANALYSIS

4.1.1 Time-domain analysis

In general, variables of time-domain analysis, or "non-spectral analysis", are relatively easy to compute and understand. The most frequently used and generally accepted time-domain variables are described below. All measurements reported in this paragraph were obtained in a population of 419 healthy subjects (291 male, 198 female), age 39.1 ± 11.3 years. All ambulatory monitoring recordings were 24 hour in duration and contained <15% time based NSI.

Time-domain variables

AVGNN: The average NN-interval. Although often this is not considered a HRV variable, the average RR-interval (or its inverse, average HR) can be used as such. Madsen[141] and Hjalmarson[93] have shown that this variable has high predictive value after myocardial infarction. This has been confirmed

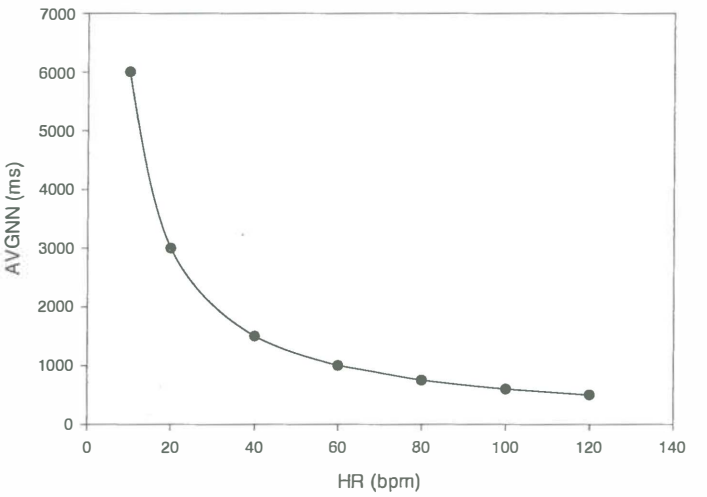


Figure 20. Relationship between heart rate and AVGNN. Obviously, the relation between heart rate and AVGNN is not linear. The difference in outcome of frequency-domain analysis as a consequence of the use of heart rate or interval power spectra, may account for the apparent differences in outcome of several HRV studies.

more recently by Copie et al, who concluded that the predischarge 24-hour mean heart rate is a strong predictor of mortality after myocardial infarction, one that can compete with left ventricular ejection fraction and HRV[47]. However, "true" HRV variables are often more sensitive to subtle specific changes in autonomic tone. Furthermore, HRV variables are superior in showing which type of fluctuations are present in a signal and hence give a better reflection of autonomic nervous system activity, something that is not possible using heart rate only. Changes in AVGNN lead to changes in all other HRV variables. In order to look at relative changes, independent of AVGNN, methods of normalisation are applied in time- as well as frequency domain. In this respect it is important to realize that the relation between heart rate and AVGNN is not linear (Figure 20). Therefore when comparing results of frequency-domain HRV analysis in particular, one should always consider whether heart rate or interval spectra were used. The importance of AVGNN is discussed in more detail in chapters 2.4.3, 5 and 8. Normal values of heart rate as well as all other HRV variables depend strongly on age and gender. In literature, normal values for heart rate are sparse. From clinical experience in ambulatory monitoring we can say these values are between 60 and 95 bpm for heart rate (AVGNN: 630 - 1000 ms) for adults. For the group of healthy subjects the mean value of AVGNN calculated over 24 hours was: 814.4 ± 102.8 ms (HR: 74.8 ± 9.12 bpm). 24-hour patterns of HRV variables, especially RR-intervals, are sometimes used to investigate the circadian variation of the autonomic nervous system [137].

SEGMENT	AVG	SD
00:00 - 00:05	avgNN1	SD1
00:05 - 00:10	avgNN2	SD2
00:10 - 00:15	avgNN3	SD3
...	...	...
23:55 - 00:00	avgNN288	SD288

Table 11. Principle of SDANN and SDNN-index computations

SDNN: The standard deviation of all NN-intervals in 24 hours. This is a measure of overall HRV. SDNN was used by Kleiger et al[156] in one of the most well-known HRV studies. In this study a decreased value of SDNN was correlated with an increased risk for sudden death after myocardial infraction. Because it measures all variability in a signal it is directly correlated to total power, computed by means of frequency-domain analysis. The circadian pattern of the standard deviation of NN-intervals shows marked similarities with the prevalence of clinical events such as ischaemic attacks [40]. Normal value: 160± 43 ms

CV: The coefficient of variance. This variable is computed as: SDNN / AVGNN, a measure of the overall HRV, corrected for effects of AVGNN. Normal value: 0.20 ± 0.04.

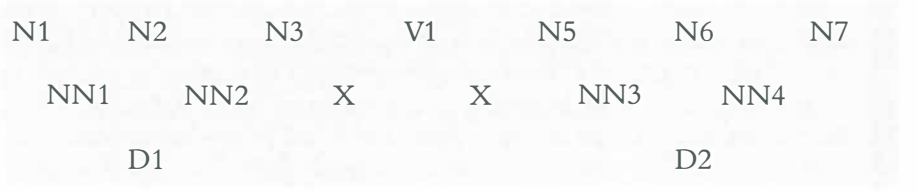
SDANN: The standard deviation of the average NN-intervals computed over 5-minute segments. Since this variable is based on differences between 5-minute segments it is a measure of long-term fluctuations[101, 182]. Dividing 24 hours into 5-minute segments results in 288 episodes. SDANN is calculated as the standard deviation of (avgNN1, avgNN2, ... avgNN288). In Table 11 the principle of this calculation is shown. Normal value: 132 ± 38 ms.

SDNN-index: To calculate this variable, first the standard deviation of all NN-intervals is computed for each 5-minute segment. Then, the average of all these standarddeviations is computed. By definition, this variable can only reflect fluctuations within 5-minute segments and therefore it focuses on short-term fluctuations. SDNN = avg(SD1,SD2,...SD288). Normal value: 68 ± 21 %.

pNN50: The percentage NN-intervals with a difference > 50 ms with the preceding NN. This variable reflects beat-to-beat changes and hence, very short-term fluctuations[38, 238]. By definition, this variable is computed over NNN-triplets. It is important to note that pNN50 is a variable based on a fixed threshold and is therefore limited in its use, especially when AVGNN changes. In adults this variable has been shown to have a strong relation with vagal activity[134]. The high heart rates in paediatric patients and some adult

patients however limit the use of pNN50 as a reliable marker of vagal activity. The recommendation of the guidelines[2] is to use rMSSD instead because of "better statistical properties". Normal value: 14.2 ± 11.2 ms.

rMSSD: The root mean square of successive differences. First the difference of a NN-interval compared to the previous is squared. Thereafter, the mean is computed. The root of this result is the rMSSD. Similar to pNN50, this variable reflects beat-to-beat variations and has been shown to be a marker of vagal activity[101, 133]. Normal value: 42.8 ± 21.3 ms



$$\text{rMSSD} = \sqrt{\frac{\sum_{i=1}^{n}(D_1)^2..(D_n)^2}{n}}$$

Figure 21. Calculation of rMSSD: In the original interval series (upper line) a sequence of N(ormal) intervals and a V(entricular) ectopic. The second line shows the resulting NN-intervals and the intervals that are excluded. In the third line D1 and D2 represent the differences between the NN-intervals.

4.1.2 Frequency-domain analysis

Frequency-domain analysis of HRV is superior to time-domain analysis with respect to the ability to divide a signal into the various modulating components. Also corrections for changes in heart rate are made more easily with frequency domain compared to time domain. Its usefulness has been demonstrated in many studies[5, 59, 175, 195]. Also visual inspection of the spectrum may be very useful. In HRV analysis, two major types of frequency-domain analysis are used, Autoregression and Fourier Transformation:

Autoregression

Autoregression (AR) analysis is a mathematical technique based on the assumption that a time series is the result of filtered "white noise" (random signal); in other words a signal can be defined by "subtracting filter-characteristics" until only white noise remains. By means of filtering a random signal, a degree of patterning emerges. After estimating the expected number of filters (parameters) that are needed to produce the actual signal from white noise, the characteristics of these filters (centre frequency and gain) can be obtained. The number of filters can be divided into pairs of 2nd order band pass filters, i.e. order of the model. However the order estimation is crucial in

this technique. Different estimation of order will produce a different outcome, which is also one of the major drawbacks of this technique. The result of AR analysis is a fluent spectrum and a spectral power density measure for which no frequency bands have to be predefined. As a technique AR is widely accepted; however, in HRV analysis it is less often used than Fourier transformation. The results of AR and Fourier transform are comparable. For reasons of standardisation and the fact the Fourier analysis is the method of choice in our research, the use of AR is not discussed further in this thesis.

Fourier transformation

The most frequently applied technique for spectral analysis of HRV is the Fourier transform, called after the French mathematician Jean Baptiste Joseph Fourier (1768 - 1830). The Fourier theorem states that every curve can be exactly reproduced by superimposing a sufficient number of harmonic waves. In other words, every curve can be constructed by piling up waves. Sinusoidal waves of different phases are "fitted" in the original signal. The extent to which such a sinus is present is called its amplitude. In order to be able to execute a Fourier transform in a mathematically reliable way, there are two major requirements for a signal that must be fulfilled:

1. The signal must be infinite. Since the time series are not, filters such as Hamming, Hanning or Parzen are usually applied to adjust for the finity of the signal. Usually, these filters have a cosine function, thus amplifying the middle part of the signal while diminishing the signal towards the end. As a result this middle part of a segment contributes more to the end-result than the start and end.
2. The signal must be stationary, meaning that the average interval must be held constant throughout a data segment.
   The result of this Fourier transform is a so-called spectrum, a plot that shows which sinusoidal waves are to which extent present in a signal. Nowadays, two major forms of this technique are used:
   - Discrete Fourier transformation (DFT), this is the original method developed by Fourier
   - Fast Fourier transformation (FFT), the popular offspring of the DFT developed by Cooley and Tukey in 1965

The advantage of FFT over DFT was its computational speed, however with increasing performance of processor technology in computers this advantage has become less important. FFT however also has a number of serious drawbacks. FFT requires that a signal also:

1. consist of a power of 2 samples;
2. be evenly spaced in time.

Since RR-intervals are not evenly spaced in time, the signal is usually resampled and then processed through the FFT(Figure 22). The effect is smoothing leading to less reliable results. When the number of data-points is increased due to resampling, the initial advantage of computational time disappears.

It is conceivable that the various techniques will perform differently and that therefore the choice of an analysis technique will influence the outcome of HRV analysis. This issue is addressed by comparing different analysis techniques using artificial as well as real ECG signals. It was found that DFT without resampling is superior to FFT using different resampling techniques, with respect to the reproduction of signal contents. An in depth explanation of the DFT analysis technique is provided by Mulder[178]. When using FFT in combination with a resampling technique for frequency-domain analysis, a decrease in heart rate will also induce the loss of high frequency power. An in-depth discussion of this topic is provided in chapter 8.

Resampling

Resampling is a technique that converts a unevenly spaced time series to an evenly spaced time series. As mentioned previously, this technique is obligatory when using FFT.

Several resampling algorithms have been used[16, 58]. An example of an often used resampling technique called linear interpolation, is shown in Figure 22: *Linear interpolation used for resampling.* Tracing I shows the original ECG signal, of which the RR-intervals are shown as vertical bars in tracing II. These vertical bars are connected though straight lines. At points evenly spaced in time, the corresponding value of this line is take as the actual value of the time series.

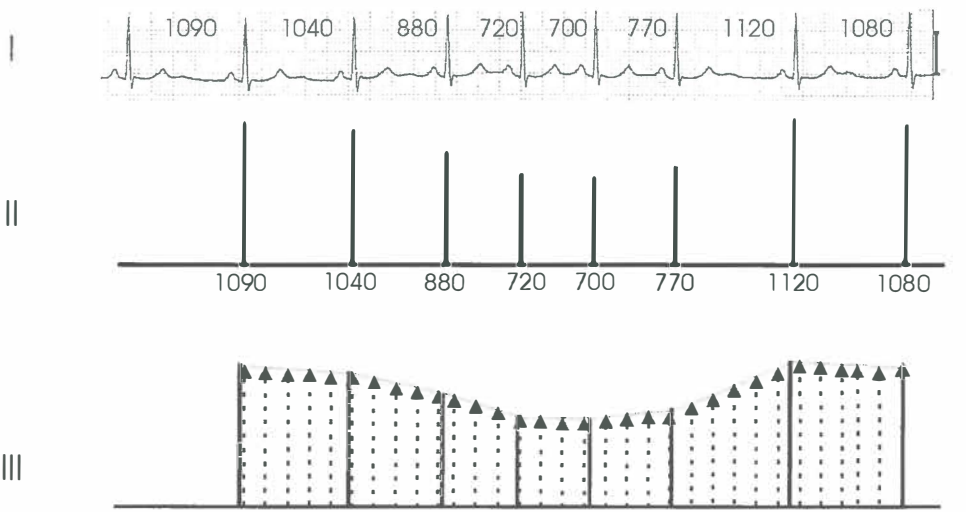


Figure 22 Linear interpolation used for resampling. Tracing I shows the original ECG signal, of which the RR-intervals are shown as vertical bars in tracing II. These vertical bars are connected though straight lines. At points evenly spaced in time, the corresponding value of this line is take as the actual value of the time series.

In step I, the original ECG tracing is shown, the numbers corresponding to the length of each RR-interval expressed in ms. In step II, these RR-intervals are rotated in a vertical direction and represented by a bar, the height of each bar equals the length of the corresponding RR-interval. Finally in step III the top of each bar is connected by a straight line and resampled by determining the height of the line at equidistant points (in this case 200 ms). In this case the resampling frequency would therefore be 5 Hz. The method described above is called linear interpolation. Hyndman and Zeelenberg showed that most of these resampling techniques have the disadvantage that they act as a low pass filter[106]. This implies that high frequency components of HRV are less well reproduced than low frequency components. DFT, requiring no resampling, is therefore preferable for HRV analysis, compared to FFT using resampling techniques. A technical description of FFT using three different resampling techniques, a comparison to DFT and the influence of average heart rate is provided in more detail in chapter 8.

Stationarity:
The requirement that a signal should be stationary implies that changes in heart rate may have a major impact on frequency-domain analysis. This is especially true, in the field of cardiology where the treatment of patients often implies alterations of heart rate (e.g. β-blocker therapy). Also daily activities during Holter monitoring lead to considerable changes in heart rate. Since non-stationarity leads to anomalies in the outcome of HRV frequency-domain analysis, a certain degree of stationarity should be warranted (see chapter 8 for a technical explanation). A measure of stationarity can be obtained by computing the relation between SDNN and TP. As shown in Equation 1, the square of the SDNN should equal the total frequency power. Based on this equation, the Parseval Index (IP) was introduced, which is defined as: IP= (square root (total frequency power) / SDNN)*100%. The Parseval index should ideally be 100%. This index is useful to ensure the amount of data exclusion due to NSI and to warrant a reasonable degree of stationarity. Data quality is ensured by only including segments that deviate less than 10% in this respect (90% < IP < 110%).

Frequency-domain analysis over a 24-hour segment:
Frequency-domain analysis can be performed over the full 24 hours of data at once or on shorter, sequential (usually 5-minute) data segments. Analysis over 24 hours in total allows the analysis of very slow fluctuations. The exact origin of these very slow fluctuations is not fully understood. Among others, the renin-angiotensin system, thermoregulation and other humoral factors have been shown to contribute to VLF and LF. In a large study, Bigger et al [23] applied 24-hour FFT in 715 patients shortly after myocardial infarction (MI) and found that power in the ULF and VLF band were strongly related to mortality (all-

cause, cardiac as well as arrhythmic death). These relations with mortality were stronger than those of higher frequency fluctuations (LF and HF). Multivariate regression analysis revealed ULF to be the strongest single component. ULF and VLF continued to show strong relations after correction for 5 established risk factors (age, New York Heart Association classification, rales in the coronary care units, left ventricular ejection fraction and ventricular arrhythmias detected by 24-hour ambulatory monitoring). Since this is a computationally very demanding tool, it has not been available for routine purposes for a long time. However with advancing technology heavy computations are feasible nowadays.

Table 12 shows the commonly used frequency-domain variables for HRV analysis.

Name	units	explanation
TP	ms^2	total power (< 0.40 Hz)
ULF	ms^2	Ultra low frequency power (< 0.0033 Hz)
VLF	ms^2	very low frequency power (0.0033 - 0.04 Hz)
LF	ms^2	low frequency power (0.04 - 0.15 Hz)
HF	ms^2	high frequency spectral power (0.15 - 0.40 Hz)
LFHF		LFHF ratio (LF / HF)
LFnu	%	normalized low frequency power (LF / (LF+HF)) *100
HFnu	%	normalized high frequency power (HF / (LF+HF))*100
ccvLF	%	component coefficient of variation ofLF (LF / AVGNN)*100
ccvHF	%	component coefficient of variation of HF (LF / AVGNN)*100

Table 12. Frequency-domain variables.

The definition of the frequency bands mentioned in Table 12 are according to the guidelines[2]. Small variations in the boundaries of LF[10, 48], HF[31, 62, 174] are frequently reported in literature. In some cases even HFLF ratio is calculated[129, 130, 220, 260] in stead of the LFHF ratio. Even though, in some specific instances there may be a valid reason for deferring from a generally accepted standard, such as in paediatric research, standardisation is valuable and deferring from these standard leads to difficulties in comparing and interpreting results. Especially the use of the HFLF ratio is confusing and should be avoided.

TP: The definition of total power is all power < 0.40 Hz. Because of its definition this variable differs depending on the duration of the data segment that is used.

ULF and VLF: The distinction between VLF and ULF is arbitrary and has no underlying physiological background. The practical reason for this boundary is the fact that the lowest frequency that can be calculated using 5-minute segments is 1/300 = 0.0033 Hz. Physiological factors that play a role in the genesis of these extremely slow fluctuations are physical activity, the circadian

pattern of the heart. In order to comply with the rule that ECG recordings should last for at least 10 times the wavelength of the lower frequency bound of an investigated component and should for stability reasons not last much longer, VLF cannot reliably be calculated from 5-minute recordings. It is therefore recommended to calculate VLF and ULF variables only from 24-hour recordings.

LF: fluctuations in the LF range are attributed to the 10 second rhythms or Mayer waves and thermoregulation, this variable is related to sympathetic fluctuations, but also vagally mediated fluctuations may occur in this range. The combined causes for the LF component have led to frequent discussions about interpretation of this variable and the use of the LFHF ratio[60].

HF: the frequency domain equivalent of rMSSD, fluctuations in heart rate > 0.15 Hz are predominantly mediated by the parasympathetic nervous system.

LFHF: the ratio of LF to HF, by some investigators considered a reflection of the balance between sympathetic and parasympathetic influence[41, 151, 189]. However this is disapproved of by others[60]. Eckberg states in his article that an large amount of literature is present to suggest that the LFHF ratio is not equivalent to the sympathovagal balance and even more that a regulatory balance between sympathetic and parasympathetic nerve does not exist. Others have questioned Eckberg's reasoning[144, 153, 217]. Nevertheless, if LF is not a perfect measure for sympathetic activity and HF is not a perfect measure for parasympathetic activity, the LFHF measure cannot be advocated to be a measure of sympathovagal balance.

The absolute variables and the LFHF variable are the basic variables. Normalized units and component coefficients are methods of normalisation (respectively to power and HR) and are discussed in chapter 2.4.3. The normalized units (LFnu and HFnu) are not independent and measure, to a certain extent, similar effects. Obviously, LFnu, HFnu and the LFHF ratio are closely related, since according to the guidelines:

LFnu = LF / (TP-VLF).
In another form this means: LFnu = LF/ (LF+HF) = 1 / (1+HF/LF)
HFnu = HF / (TP-VLF).
In another form this means: HFnu = HF / (LF+HF) = 1 / (1+LF/HF)
LFHF = LF / HF

The formulas depicted above clearly show the relationship between the three variables.

4.1.3 Geometric analysis

Geometric analysis is a form of analysis in which the RR-interval series is primarily converted to a graphical form. This qualitative approach can be a very useful and fast way to obtain a global impression of the RR-interval distribution. It might be argued that judging graphs is a subjective form of analysis and may therefore be – more than other methods – prone to observer bias or higher inter-observer variability. In order to quantify the geometrical RR-interval distributions, several variables have been introduced[143, 148]. These geometric variables have been shown to be more robust in imperfect recordings and less sensitive to noise. The major disadvantage of the geometric methods is that they all require substantial amount of data to be applied reliably. Consequently these methods cannot be applied in short recordings such as physiological experiments or in autonomic function tests. The following geometric variables can be distinguished:

1. Triangular index: The integral or total number of all NN-intervals, divided by the height of a histogram (the maximum number of RR-intervals that is contained in a single bin).
2. TINN: Triangular interpolation of the NN-interval histogram. This is computed by taking the maximum point of the NN histogram and creating a triangle by fitting a line downwards to the baseline, using the least squares method. The minimum and maximum cross-points obtained in this way are subtracted. The result is the TINN measure.

These two quantitative variables are simple and global variables of overall variability[54]. They show a strong relationship with SDNN. However, they are believed to be less sensitive to misdetection and classification errors that may occur in ECG analysis[147]. It should be noted that the resolution of the histogram is critical. For standardisation reasons it is advised to calculate these variables using a resolution of 7.8125 ms, which corresponds to the sampling frequency of most commercially available ambulatory monitoring systems.

Poincaré plots:
Another way of presenting RR-intervals is a so-called Poincaré or Lorenz plot. The mathematician Henri Poincaré (1854-1912) invented the notion of an abstract dynamical system and the field of topology. In the field of cardiology this technique is used by plotting RR-intervals against the preceding RR-interval. In other words this technique focuses on magnitude and correlation, while the timescale is represented by the sequence of the dots, a feature that is not visible to the eye, but available in computer analysis. In Figure 23 a Poincaré plot is shown of a 3-hour Holter recording. Representing all RR-intervals in 24 hours using a Poincaré plot may reveal disturbances which are less readily

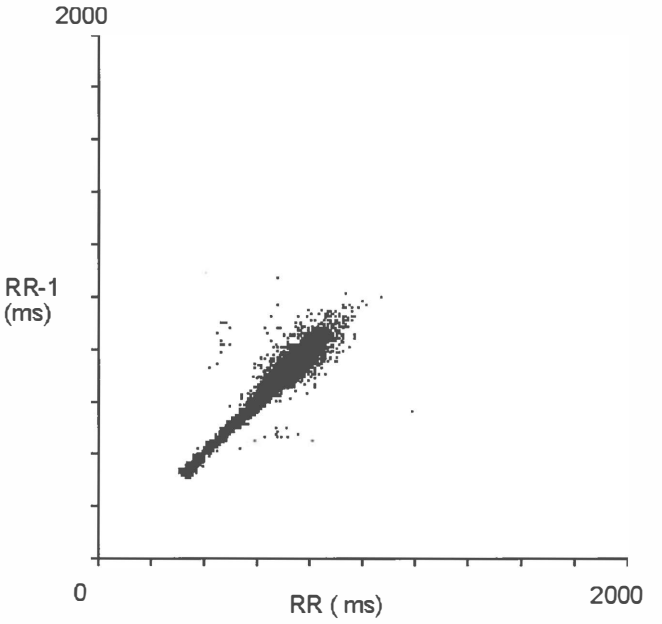


Figure 23. Poincaré plot

reflected in conventional HRV variables. A classification method, depending on shape, has been proposed in order to classify Poincaré plots[264]. In subjects with preserved HRV the Poincaré plot shows a widely scattered graph, while a depressed HRV is characterized by a compact pattern. This visual inspection of Poincaré plots has been applied successfully in order to discriminate between normals and congestive heart failure patients[265], between the functional class of congestive heart failure patients with low (I and II) and high (II and IV) New York Heart Association classification[112] and to identify patients at risk for sudden death in a severe congestive heart failure population[262]. Furthermore, in a mild congestive heart failure population it was shown that Poincaré plots have an independent prognostic value compared to other variables of HRV[39, 43]. More recently quantification methods for Poincaré plots have been proposed[94, 111]. Although these methods are promising, they are still experimental. These types of computations resemble to a certain extent the non-linear approaches of HRV (see chapter 4.2). In all of the geometric techniques it is important to realize whether or not NSI are included. Although more clearly seen and more emphasized in spectral techniques, in- or excluding NSI will lead to differences in the various graphical representations of RR-intervals.

4.2 NON-LINEAR ANALYSIS

Although assessing non-linear techniques is not the primary focus of this thesis, a methodological summary is presented here to explain the position of these techniques. Therefore this paragraph provides an overview of the currently used non-linear analysis methods. Although a relatively small number of studies is available and more validation work needs to be done, non-linear techniques are very promising and should be developed further.

4.2.1 Introduction

What is nonlinearity ? Linearity and nonlinearity are concepts that have their origin in mathematics. Using techniques from the field of physics requires some understanding of basic concepts. In geometry, linearity refers to Euclidean objects: lines, planes, cubes, etc. These objects look the same no matter how we examine them. A non-linear object, a sphere for example, looks different on different scales. Furthermore, while we can enumerate linear objects(lines planes), non-linear ones (such as spheres) are non-enumerable. Mathematically speaking, in linear systems the output of a function $f_{(x,y)}$ is proportional to the input of the model, while in non-linear systems (the negation of linear) the result of the function may be out of proportion to the input of the model. Because of greater simplicity linear models are more frequently used than non-linear models, however no model of a natural system is truly linear. Dynamical systems can be divided into two types: deterministic and stochastic. A deterministic system is a system in which there is just one consequent to every state, this in contrast to a stochastic or random system in which a certain state of the system may have more than just one consequent.

The coherence of these models is shown in Figure 24.

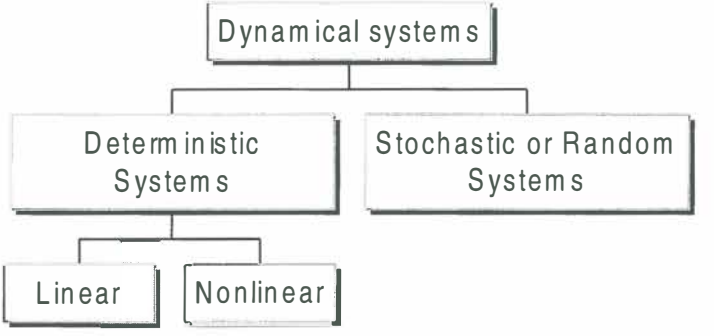


Figure 24. The coherence of models in system dynamics.

Non-linear systems have been shown to exhibit surprising and complex effects. One of these effects is chaotic behaviour. A definition of chaos is an effectively unpredictable long-time behaviour arising in a deterministic dynamical system, because of sensitivity to initial conditions. In other words a small change in starting point may lead to large differences in outcome. As a consequence of this time series may appear irregular and disorderly. Dynamical systems have a tendency to "settle down" at certain points in time. The point that the system approaches is called an attractor. In chaotic systems this is called a chaotic attractor, while in fractal signals this is called a strange attractor. The correlation dimension of a signal is a measure of the size of an attractor. Fractal dimension, Lyapunov exponents and entropy are measures of non-linear models, that describe the degree of complexity.

In various studies linear techniques, time- as well as frequency domain, have proven to be successful in identifying patients at risk [65, 122, 234]. However there are also a number of instances where linear models have failed to discriminate between groups, or have been less successful compared to non-linear methods[39, 251]. There is a theoretical basis for the assumption that this might partially be explained by the data analysis technique. In non-linear analysis ectopic beats are often included, thus leading to at least less selection bias. Non-linear measures, theoretical & technical considerations and a comparison to linear techniques are described in the following paragraphs. Non-linear analysis is a relatively new field in physics and there is certainly no large experience in the application of these analysis forms in HRV. In clinical practice this means that guidelines, technical recommendations and certainly a consensus about the different methods that should and should not be used are not readily available. However a number of basic principles that are valid for linear analysis also apply for non-linear analysis of HRV.

4.2.2 Non-linear methods and variables

An excellent technical review of the various methods is given by Mansier and co-workers [159]. In this chapter definitions and measures of non-linear analysis are presented.

Dimension

The concept of dimension plays an important role in the understanding of non-linear analysis.

When considering a number of points in phase space, a cloud emerges. From such a cloud (or its 2D equivalent, a Poincaré plot) the dimension can be computed. For simple forms the Euclidean or embedding dimension is simple: for a line it is 1, for a plane it is 2, while for a cube the dimension is 3. However, theoretically speaking, if the one would draw a line with a changing direction

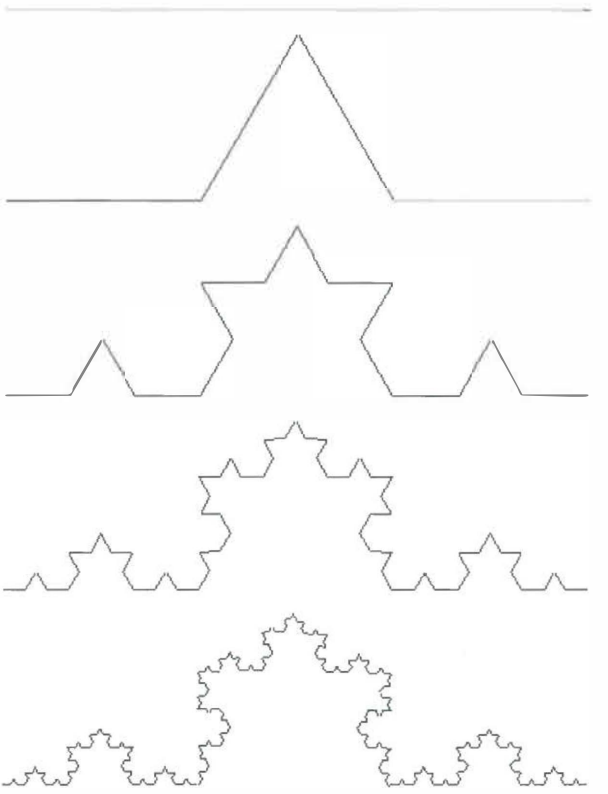


Figure 25. The Koch snowflake. This form is self similar and fractal of nature and can be produced by replacing each straight line by the basic component as shown in the second tracing.

long enough, the line would become a plane. Therefore the dimension of this object has changed. There are different ways of computing a dimension, however the basic "problem" is the same. The result of the measurement is dependent on the measurement scale. Probably the best-known demonstration of this is the calculating the length of a coastline. If the coastline is measured using a coarse measurement scale the total length will be less compared to a measurement using a fine measurement scale. After all, using the fine scale will cause small bays to be included in the measurements. This was shown by Mandelbrot in his article: How long is the coast of Britain?[155] One way to access this problem is to compute the Hausdorff-Besicovitch dimension. This is defined as the quotient of the log change in object size and the log change in measurement scale. For a simple line this would be 1, since multiplying a line with a length of 1 using the factor 2 results in a line with a length of 2; therefore the Hausdorff-Besicovitch dimension is log 2 / log 2 = 1. Applying the same formula to a plane this results in a plane that is 4 times the original size. The resulting Hausdorff-Besicovitch dimension = log4 / log2 = 2. The application

of this formula to a well-know form, the so-called Koch snowflake, results in a different outcome. The basis of the Koch snowflake is shown in Figure 25. Consider a replacement of each line as in Figure 25-1 by a form as in Figure 25-2. The resize factor 3 would result in a factor 4 larger snowflake, since one of the original snowflakes can be placed on each of the 4 segments. This process is repeated 4 times in Figure 25. The resulting Hausdorff-Besicovitch dimension = log4 / log3 = 1.26 and therefore the Koch snowflake is a fractal. The fractal dimension may be considered an interpolation between topological dimensions.

Fractals: According to the definition by Mandelbrot, all sets of which the Hausdorff-Besicovitch dimension exceeds the topological dimension are fractal. A more popular definition of a fractal is a fragmented shape that can be subdivided in parts, each of which is a reduced-size copy of the whole. Fractals are selfsimilar and independent of scale. Different algorithms are developed to assess the "fractal dimension" of a certain set. In HRV analysis the Grasberger-Procaccia algorithm is frequently used [97,216]. This algorithm calculates a measure of the geometry of a cloud of points and is sometimes applied in Poincaré plots.

Lyapunov exponents: These exponents calculate the average local rate of divergence of neighbouring trajectories. In a system there are as many Lyapunov exponents as there are dimensions in phase space. Of these the largest is usually the most important. Positive Lyapunov exponents in deterministic systems, indicate sensitive dependence on initial conditions and implies an exponential growth of perturbations. Therefore, in a deterministic system, a positive Lyapunov exponent can be used as a definition of a chaotic state.

Entropy: Entropy is the measure of disorder in a system. When entropy increases, disorder increases. A well-know measure of entropy in physics is Shannon entropy.

Complexity: While chaos describes how simple things can become really complex, complexity describes how complex systems can result in simple behaviour. Kolmogorov's notion of complexity is a measure of randomness, one that is closely related to Shannon entropy.

Symbolic dynamics: Starting off with a definition, in symbolic dynamics RR-intervals are coded according to length. An example of a definition is given in Table 13.

Applying this definition, the RR-interval sequence is replaced by a sequence of numbers. As a second step this sequence is scanned for the presence of so-called words; it is scanned for the presence of certain sequences. Finally the

symbol	RR-interval
A	RR-interval =< 0.8* AVGNN
B	0.8* AVGNN < RR-interval =< AVGNN
C	AVGNN < RR-interval =< 1.2 * AVGNN
D	1.2*AVGNN < RR-interval

Table 13. Example of a definition in symbolic dynamics.

distribution of words is studied. Although this is a coarse method it has proven to be useful at least in one study in identifying high-risk patients after myocardial infarction[251].

α-scaling index

The alpha scaling index is a technique that computes an average vector of all points in a Poincaré plot compared to all the other points[213, 214]. Unpublished data suggests that this is a promising and useful non-linear technique.

5.
NORMAL VALUES OF HRV

5.1 INTRODUCTION

For HRV to be applied in clinical practice, it is essential to define normal values. Similar to heart rate, a relation between HRV (normal) values, age and gender is conceivable. Moreover a relation with heart rate per se is also feasible. Variance of HRV in normal subjects determines what can be considered normal values. However, normal values are hard to define, since they possibly vary with age and gender but certainly, in case of frequency-domain variables, with the computational method. Several investigators have studied the relationship between heart rate, age and HRV[52, 130, 193, 248]. Although all studies show a decrease of HRV values with both increasing heart rate and age, no agreement exists on gender effects. Ryan and co-workers[208] conclude that HRV differences between men and women exist, while other investigators claim that differences in HRV variables because of gender can be fully explained by differences in heart rate. By far the largest study on determinants of HRV was performed by Tsuji et al, in recordings from the Framingham study[233]. They investigated 2722 subjects, using multiple regression analysis to identify independent determinants of HRV. Higher heart rate, older age, female gender, smoking, the prevalence of ectopic beats and the consumption of 3 cups of coffee or more per day, were identified as determinants of HRV, heart rate and age being the strongest determinants. Unfortunately, this analysis was restricted to only the first 2 hours of the recording at most. Recently Umetani et al[239] studied the relationship between age, gender and time-domain variables of HRV in a group of subjects, comprising 9 decades. These authors showed that the decrease of HRV values with age depends on the type of variable that is studied and that ageing lowers HRV variables below values known from heart failure studies to be associated with increased mortality. Also, these authors showed the disappearance of gender-related differences above the age of 50 years, a finding that was also reported by Stein et al[223]. In a very young group, age 3 days to 14 years, Massin et al.[160] established normal values for time- as well as frequency-domain variables and demonstrated the independence of age- and heart rate-related decrease of HRV values. Normal values are presented in the guidelines, however, these are based on a small number of subjects and not adjusted for age, gender, heart rate etc. Though the above studies are valuable, most do not report both time- and frequency-domain values, recording duration is not always standardized and FFT is mostly used for frequency-domain analysis, while in our centre DFT is the method of choice (see chapter 8). Since the computational method of frequency-domain analysis influences the actual outcome, we investigated normal values of HRV, time as well as frequency domain, and their dependence on heart rate, gender and age, in a group of 419 healthy subjects; 291 males, 198 females, mean age: 39.1 ± 11.3 years. Only subjects without a history of any major disease were allowed in the study and physical examination was performed on all subjects. Also, no abnormalities indicative of structural heart

disease were allowed during ambulatory monitoring. Only ambulatory monitoring recordings containing over 23.5 hours were used, all of which contained <15% time-based NSI. Statistical analysis was performed using SPSS version 7.5. Correlation coefficients were computed using Pearson's correlation coefficients, while Spearman's rho was used for non-normal distributed variables (marked *).

5.2 Normal values of time-domain variables, relation to gender and age

An inverse relation exists between age and time-domain HRV variables, as demonstrated in Figure 26 for SDNN and rMSSD. The strength of this relation is shown in Table 14. In Figure 27, a clear relationship is shown between

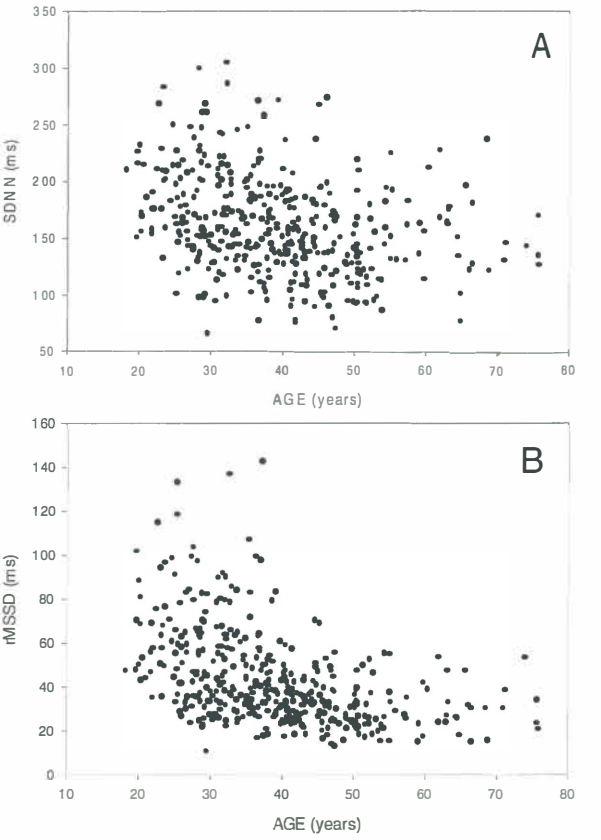


Figure 26. SDNN (panel A) and rMSSD (panel B) in relation to age. Both HRV variables decrease with increasing age.

different time-domain variables and heart rate. Also in this figure the difference between heart rate and AVGNN is shown in relation to rMSSD. There is a clear similarity, however the relationship with AVGNN is more skewed to the right. In Table 15 and Table 16 the values of time-domain HRV variables are shown for male and female subjects respectively. These tables are divided into four consecutive decades of age.

HRV Variable	AVGNN	AGE
SDNN	0.63	-0.30
SDANN	0.51	-0.29
SDNNindex	0.65	-0.45
rMSSD*	0.53	-0.51
PNN50*	0.57	-0.53
CV	0.21	-0.36

Table 14. Correlation coefficients between HRV and AVGNN / age.

Multiple regression analysis of this data revealed a dependence on heart rate and age, however no significant relation with gender was observed.

Male Age	n	AVGNN	SDNN	CV	SDNNindex	SDANN	rMSSD	pNN50
20-30	57	862 ± 95	199 ± 40	.23 ± .04	86 ± 18	167 ± 35	63 ± 23	24.2 ± 11.7
30-40	86	843 ± 121	175 ± 42	.21 ± .04	80 ± 22	145 ± 38	51 ± 23	18.5 ± 11.6
40-50	91	814 ± 96	148 ± 39	.18 ± .04	63 ± 15	122 ± 33	34 ± 11	9.2 ± 6.6
50-60	46	828 ± 103	143 ± 36	.17 ± .04	54 ± 14	119 ± 35	29 ± 11	7.3 ± 6.7

Table 15. Normal values for male subjects sorted by age.

Female Age	n	AVGNN	SDNN	CV	SDNNindex	SDANN	rMSSD	pNN50
20-30	37	764 ± 74	155 ± 33	.20 ± .03	72 ± 18	125 ± 30	50 ± 22	18.9 ± 10.5
30-40	48	754 ± 82	146 ± 35	.19 ± .04	63 ± 18	120 ± 32	40 ± 21	13.9 ± 11.2
40-50	21	776 ± 76	145 ± 35	.19 ± .03	54 ± 28	118 ± 28	31 ± 10	8.2 ± 6.6
50-60	11	760 ± 70	123 ± 19	.16 ± .02	53 ± 9	99 ± 21	33 ± 8	9.5 ± 7.8

Table 16. Normal values for female subjects sorted by age.

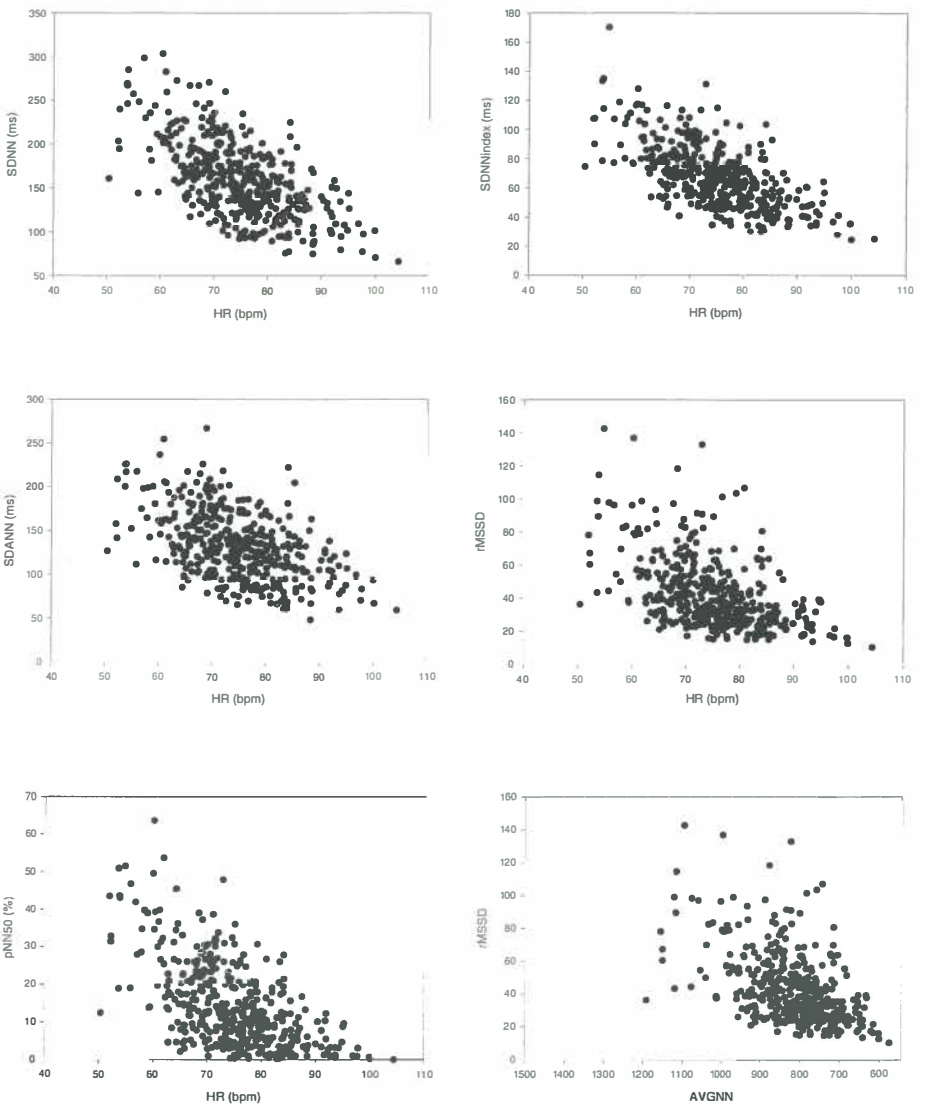


Figure 27. Time-domain variables in relation to heart rate. All non-normalized time-domain variables decrease with increasing heart rate. The lower right plot shows the relationship between rMSSD and AVGNN instead of heart rate. This plot, when compared to the plot above shows the difference between AVGNN and heart rate in relation to rMSSD. Although there is a clear similarity, the relationship with AVGNN is more skewed to the right.

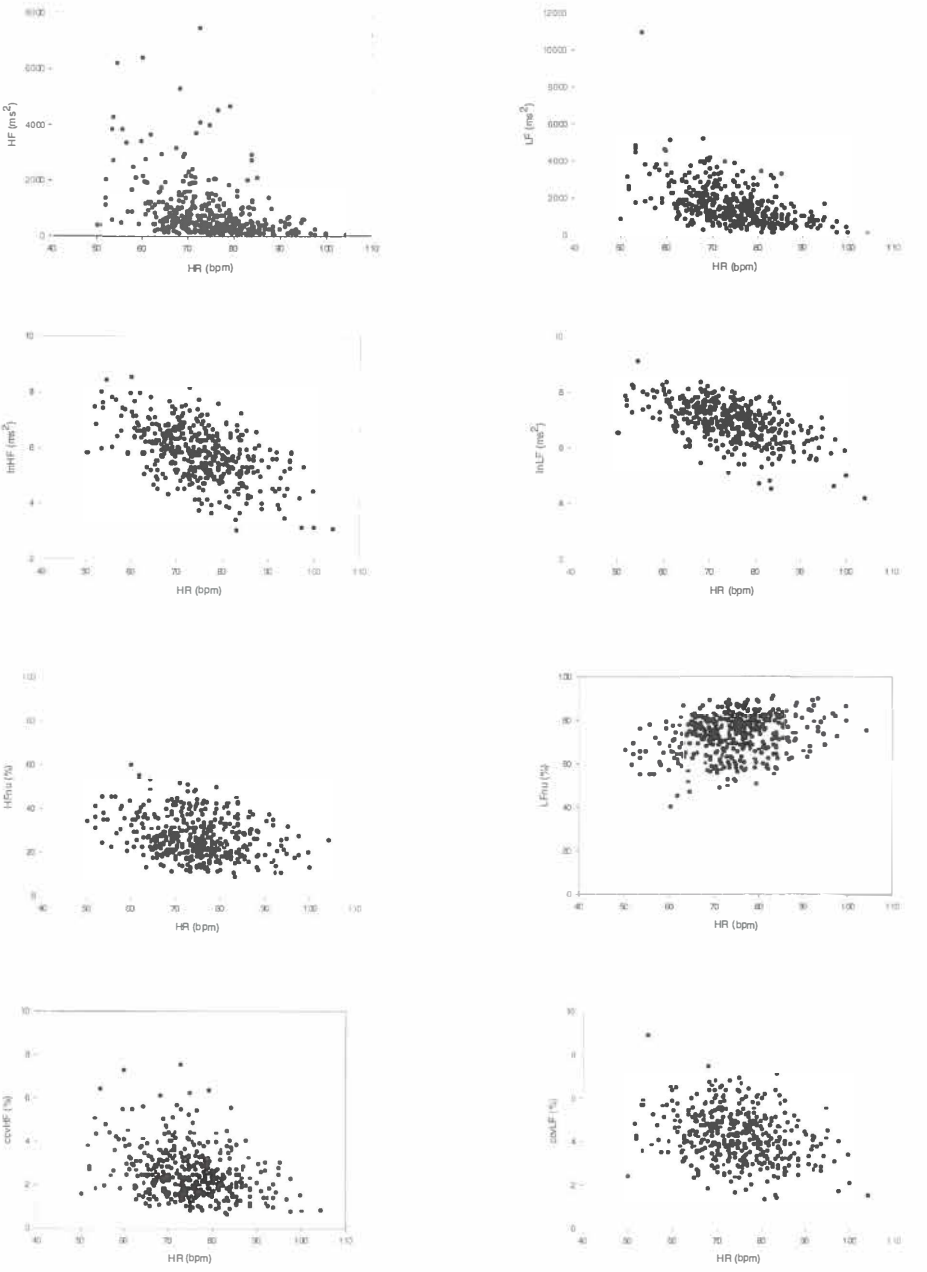


Figure 28. Frequency-domain variables in relation to heart rate. A clear decrease of all frequency domain variables except for LFnu is seen with an increasing heart rate.

5.3 NORMAL VALUES OF FREQUENCY-DOMAIN VARIABLES, RELATION TO GENDER AND AGE

The relation with age, AVGNN and gender was also established for frequency-domain variables of HRV (absolute, normalized and CCV variables). Figure 28 shows the effect of heart rate on the LF and HF components of HRV. In Figure 29, the decline of LF in relationship to age is shown, while the same relationship with HF is demonstrated in panel B, leading to a larger decrease of HRV in early years and more or less levelling off at a higher age. Correlation coefficients between HRV variables on the one hand and age and AVGNN on the other are shown. Table 17 contains the correlation coefficients for the various frequency-domain variables with AVGNN as well as age. All relations were significant ($p < 0.01$). Like in time-domain analysis, multiple regression analysis of this data revealed a dependence on heart rate and age, however no significant relation with gender was observed. In Table 18 and Table 19 the average values for male as well as female healthy subjects are shown, computed per age decade.

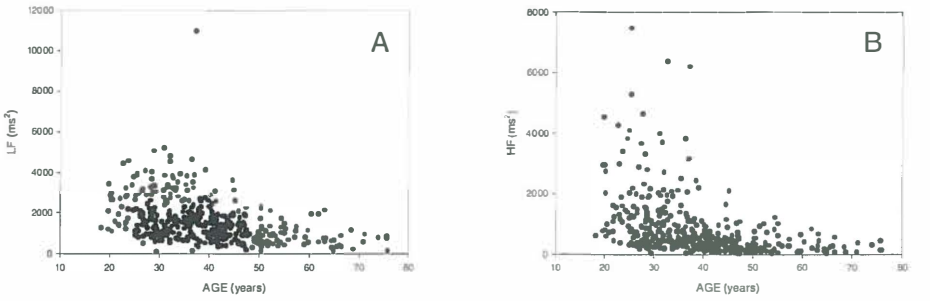


Figure 29. Frequency-domain variables LF (panel A) and HF (panel B) in relation to age. Both variables decrease with increasing age. A strong decrease is seen especially during early years.

Variable	AVGNN	AGE
TP*	.65	-.47
LF*	.54	-.49
HF*	.45	-.57
LFHF*	-.23	.36
LFnu*	-.24	.39
HFnu*	.24	-.39
ccvLF	.26	-.56
ccvHF*	.29	-.62

Table 17. Correlation coefficients between frequency-domain variables and AVGNN / age.

Male Age	n	TP	LF	HF	LFHF	LFnu	HFnu	ccvLF	ccvHF
20-30	57	9152±3684	2467±1002	1608±1354	3.6±1.6	68.3±9.2	31.7±9.2	5.1±0.8	5.1±1.2
30-40	86	7963±4831	2293±1412	1014±960	4.2±1.8	73.3±8.1	26.7±8.1	5.0±1.1	2.9±1.0
40-50	91	4840±2309	1383±675	395±322	5.4±2.1	78.9±6.3	21.1±6.3	4.0±0.8	2.0±0.6
50-60	46	3709±1937	921±520	293±283	5.5±2.1	78.0±7.1	22.0±7.1	3.2±0.8	1.6±0.6

Table 18. Normal values for male subjects sorted by age.

Female Age	n	TP	LF	HF	LFHF	LFnu	HFnu	ccvLF	ccvHF
20-30	37	6211 ± 3214	1632 ± 813	1211 ± 1146	3.0 ± 1.3	65.8 ± 8.7	34.2 ± 8.7	4.6 ± 0.8	3.3 ± 1.1
30-40	48	4901 ± 3110	1257 ± 741	783 ± 1052	3.5 ± 1.8	68.6 ± 9.4	31.4 ± 9.4	4.1 ± 0.9	2.7 ± 1.2
40-50	21	3810 ± 2080	942 ± 485	379 ± 311	4.5 ± 1.9	74.1 ± 6.8	25.9 ± 6.8	3.4 ± 0.6	1.9 ± 0.7
50-60	11	3212 ± 1004	786 ± 191	373 ± 245	4.0 ± 1.4	71.7 ± 7.6	28.3 ± 7.6	3.4 ± 0.4	2.0 ± 0.5

Table 19. Normal values for female subjects sorted by age.

Discussion:

Time-domain analysis: A decrease of HRV variables was observed with an increasing age. The CV variable (SDNN / AVGNN), a value that is corrected for heart rate also showed a decrease with age. This demonstrates that the decrease of CV, is not the consequence of increased heart rate. In panel A of Figure 26 the decline of SDNN in relationship with age is shown, while in panel B the same relationship with rMSSD is demonstrated, leading to larger decrease of HRV in early age and more or less levelling off at higher age. This finding is consistent with findings of other authors[239]. Considering the group as a whole, gender related differences of time-domain HRV variables are the consequence of differences in heart rate. The absence of gender-related differences is confirmed by others in normal subjects[34, 267] and patients with diabetes mellitus[34]. However, since a difference in heart rate exists based on gender, normal values should nevertheless be specified according to gender and age. The relationship of HRV variables with age depends on the type of variable. Similar to other studies[239] decreased HRV values may fall below cut-off points used for increased risk of mortality. This also emphasizes the need for age- and gender-matched controls.

Frequency-domain analysis: With respect to frequency-domain analysis it is important to realize that normal values depend on the computational method used. Furthermore, the amount of NSI accepted for analysis, the amount of non-stationarity accepted for analysis, the type of resampling and the spectral analysis method used, are all of influence on the values obtained. Therefore, cut-off values must be determined in each study and may vary, depending on the specific patient population that is observed. For example, in studies focusing on normal subjects a maximum amount of 5-10% of NSI is often used, while using these cut-off values in a heart failure population may lead to large portions of excluded signal and therefore to a selection bias. When considering heart rate, a clear decrease of most frequency-domain variables is seen with an increasing heart rate. All frequency-domain variables decline more in early years and stabilize during later years. In male subjects TP decreases about 5000 ms^2 in 30 years. Because LF and HF are non normally distributed variables, often a log transformation is applied in order to obtain a normal distribution. The lnHF and lnHF variables therefore show a better relation with heart rate than their non-transformed counterparts as shown in Figure 28. HFnu also decreases with an increasing heart rate, while LFnu increases with an increasing heart rate. This is the consequence of a shift in sympathovagal balance towards sympathetic predominance. The correlation coefficients of LFnu and HFnu are exactly opposite, since these variables are each other's complement. It is notable that, similar to CV in time domain, even the CCV variables of HRV show a significant relation with AVGNN, even though these are heart rate-corrected variables. Frequency-domain variables are also inversely related to age. Again a different relation is observed for the different HRV variables. As in time-domain analysis, gender-related differences are the consequence of differences in heart rate.

In conclusion: most values of HRV decline with age and heart rate. Differences in gender are the consequence of differences in heart rate. The relationship between age and HRV variables depends on the type of variable that is studied. These findings are consistent with other findings in literature. In this chapter normal values of time- and frequency-domain analysis of HRV are presented, that can be used for clinical as well as research purposes. For practical purposes these normal values are presented, sorted by gender and age. Limitation: as previously described, normal values depend on several cut-off values used in a study. Normal values should be calculated using exactly the same cut-off values such as amount of NSI on an age- and gender-matched control group.

6.
INFLUENCE OF RECORDING DURATION

Many technical aspects may influence the outcome of HRV analysis. One of these aspects is recording duration. The potential influence of recording duration on the outcome of HRV can theoretically be attributed to two causes. In the first place mathematical properties of a variables may change if the duration of the data-segment that is used, varies. However, all time domain HRV variables are by definition mathematically independent of the duration of a data-segment. In frequency-domain analysis, the lowest frequency component that can be computed is dependent on the duration of the data segment. This factor may play a role when short data segments are used. When analysing 24-hour ambulatory monitoring recordings frequency-domain analysis is mostly applied in fixed-duration (e.g. 5-minute) data segments and because of this fixed duration, mathematical properties of frequency-domain variables do not vary. Therefore, when using the methods as described above the mathematical properties can be neglected. As a second cause the hookup-time of ambulatory monitoring should be considered. In clinical practice, hook-up times of ambulatory monitoring recordings are not randomly divided over time, but a Holter recording is usually started during day hours. Holter recordings are often ended prematurely because the recorder is needed for another patient. Also patients cannot take a bath or a shower when carrying a Holter and by the end of a registration patients often feel the need to stop the recording and take care of personal hygiene. This means that recordings less than 24 hours will primarily miss "daytime hours", that is early morning hours. Therefore, shorter recordings will more often lack sympathetically dominated data. It is likely that the effect of recording duration is not similar for different groups of patients. If total variability is low, variability during the day is also likely to be less pronounced. In other words, in patients with depressed HRV, duration-related changes will probably be less marked than in normal subjects. The guidelines advise that a Holter recording used for HRV analysis should last at least 18 hours including the whole night, since long-term HRV fluctuations exist mainly due to day-night differences. Furthermore, it is stated that "It is inappropriate to compare time-domain variables, especially those expressing overall HRV, obtained from recordings of different durations." Although these statements are undoubtedly true, little literature is available on this issue, and little data-driven evidence is presented. In a post myocardial infarction population, Malik et al compared two groups, one with and one without cardiovascular events. HRV values obtained from 24-hour recordings were able to distinguish significantly between the two groups. However, The predictive value of HRV obtained from single 1-hour recordings was only in a limited number of hours able to distinguish between the same two groups[145], while other single 1-hour recordings failed to discriminate. From this study it was concluded that arbitrarily chosen 1-hour segments cannot replace the 24-hour average of HRV.

In order to test recording-duration related changes of HRV we tested three groups:

1. Normal subjects (n = 24, mean age 53 ± 3 years, physical examination and ambulatory monitoring revealed no abnormalities, no history of any major disease)
2. Congestive heart failure patients (CHF, n= 24, mean age 56 ± 3 years New York Heart Association class II - III)
3. Patients with proven ischaemic heart disease (IHD, n=21, mean age 63 ± 2 years, stable angina pectoris, >= 4 episodes of myocardial ischaemia during 48-hour ambulatory monitoring)

Recordings were made using Marquette series 8500 recorders. Only ambulatory monitoring recordings containing 24 hours of data were used. All recordings were started during daytime hours and no selection with respect to start time was made. The ECG was manually analysed by an experienced Holter technician who identified noisy episodes and ectopic beats using a Marquette Laser Holter system (Series 8000 XP). Thereafter, the RR-interval series was transferred to the COHORT system, a locally developed software package (written in DELPHI™ and FOXPRO®) used as a post processor for detailed HRV analysis. Time-domain analysis was performed over the full 24 hours, while frequency-domain analysis was performed over 5-minute segments using Discrete Fourier Transformation. Only data segments containing <5% time-based non sinus intervals and a Parseval index deviating <10% were used for analysis. The resulting data were used to obtain the 24-hour average, after which the last hour of each RR-interval series was removed and the HRV analysis was performed again. Student-t test was used to analyse the differences due to duration reduction. P values < 0.05 were considered to be statistically significant.

In Figure 30 the influence of recording duration on the various time-domain variables in the group of normal subjects is shown. The asterisk in Figure 30 depicts the first hour that changed significantly from the 24-hour average for each of the time-domain variables in normal subjects. This effect proved to be similar for AVGNN, SDNN-index and rMSSD on the one hand (variables of relatively short-term variations), and for SDNN and SDANN (variables of relatively long-term variations) on the other hand. In the AVGNN group all variables decrease, after which an increase is demonstrated. In SDNN and SDANN however, a continuous decrease of the variable is seen. Comparison per patient of the 24-hour results to the other recordings durations showed that AVGNN already changes significantly if the recording duration is decreased to 23 hours.

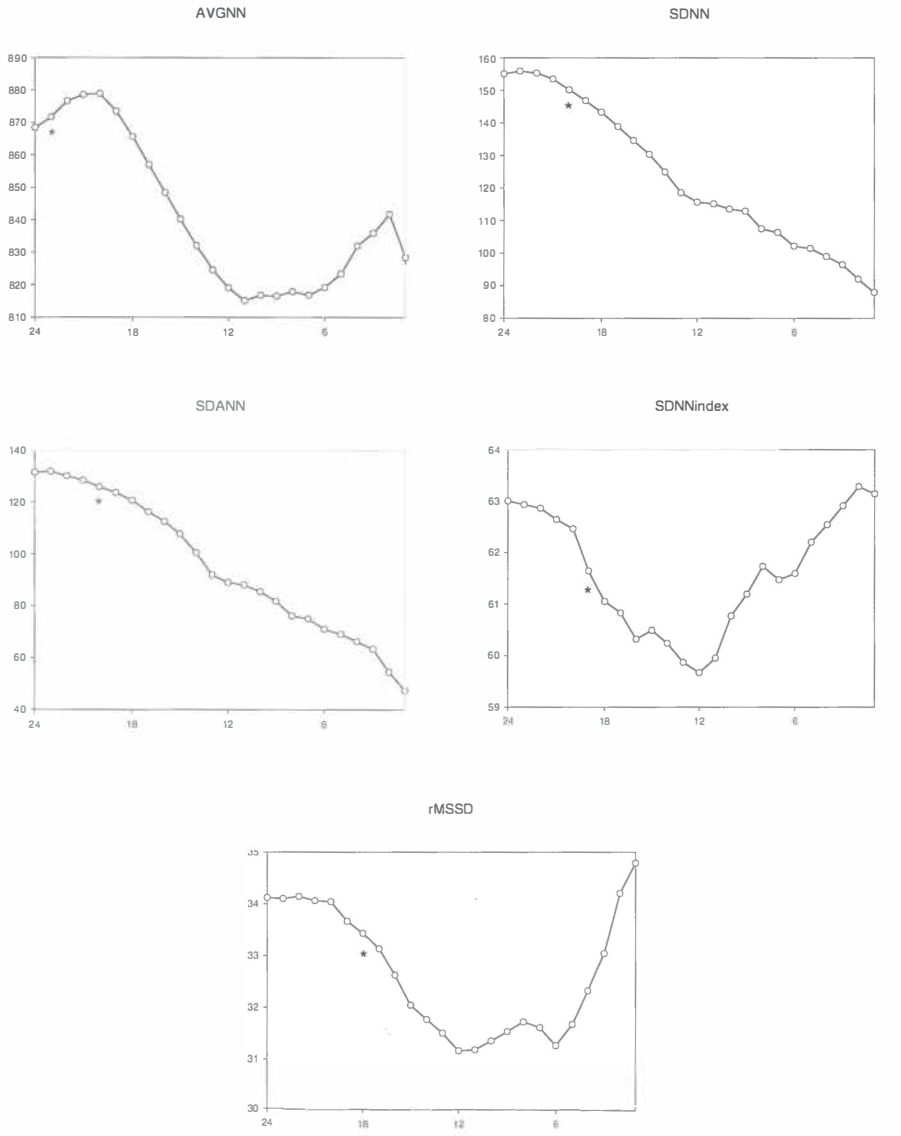


*Figure 30. Influence of recording duration on time-domain variables. The * marks the first point where a decrease in recording duration leads to a significant change in the HRV variable .*

Variable	Normals	IHD	CHF
AVGNN	23	23	23
SDNN	20	20	16
SDANN	20	19	23
SDNNindex	19	20	19
rMSSD	18		11
LF	14	14	18
HF	15	16	18
TP	19	18	19
LFHF	17	17	17
LFnu	17	15	17
HFnu	17	15	17
ccvLF	2	8	7
ccvHF		17	18

Table 20. The first hour that demonstrated a significant change compared to the 24-hour average when recording duration was reduced in steps of 1 hour. The three groups are shown: normals, congestive heart failure (CHF) and ischaemic heart disease (IHD). The blank values demonstrate that no significant change occurred. The first column of this table shows the hours that are marked by an asteriks in Figure 30 and Figure 33.

Variable	Normals	IHD	CHF
AVGNN	7.4	11.1	12
SDNN	43.8	39.7	44.2
SDANN	64.2	70.4	65.6
SDNNindex	5.7	5.9	6.9
rMSSD	10.6	12.3	19.5
TP	15.5	20.3	17.0
LF	15.0	16.2	15.3
HF	32.4	43.2	39.0
LFHF	19.6	21.8	13.3
LFnu	5.9	5.3	5.3
HFnu	19.2	18.0	17.0
ccvLF	9.4	12.4	11.7
ccvHF	8.4	8.2	8.3

*Table 21. The maximum amount of change that occurred as a reduction of recording duration expressed as a percentage of the initial 24-hour value : ((max-min)/initial value)*100%.*

Table 20 shows when significant recording duration changes occur in the three patient groups, while the amount of change is shown in Table 21.

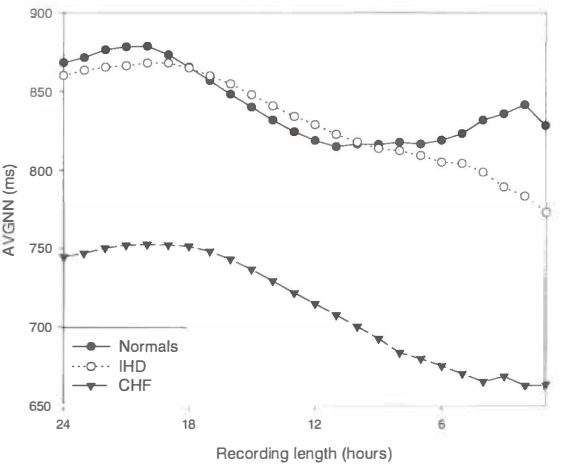


Figure 31. Effect of recording duration on AVGNN in the 3 different groups: Normals (closed circles), congestive heart failure patients (open circles) and patients with ischaemic heart disease (triangles).

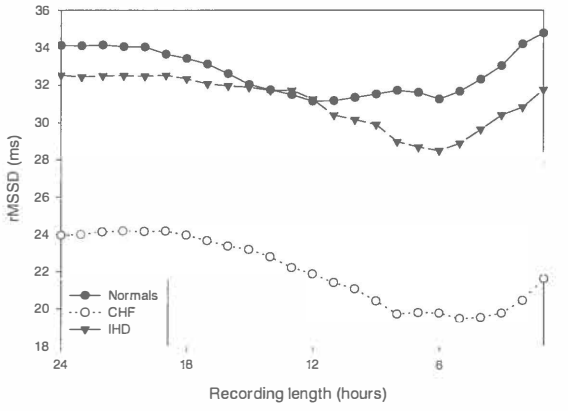


Figure 32. Effect of recording duration on rMSSD in the 3 different groups: Normal subjects (closed circles), congestive heart failure patients (open circles) and patients with ischaemic heart disease (triangles).

In Figure 31 and Figure 32 the recording duration-related changes for the three groups are shown for AVGNN and rMSSD.

In Figure 33 the influence of recording duration on the various frequency-domain variables is shown, for the 24 normal subjects. Again the first hour to change significantly is marked with an asterisk. It is noteworthy that the upper left plot showing recording duration effects on TP is strikingly similar to the SDNNindex plot from Figure 30. This is explained by the fact that SDNN is calculated over the entire recording in toto, while TP as well as SDNNindex are based on 5-minute calculations. Another factor that must be kept in mind when comparing time domain with frequency domain variables in this respect is that in frequency domain analysis an amount of 5-minute episodes may be excluded due to NSI while in time domain calculations only the NSI episode itself is discarded.

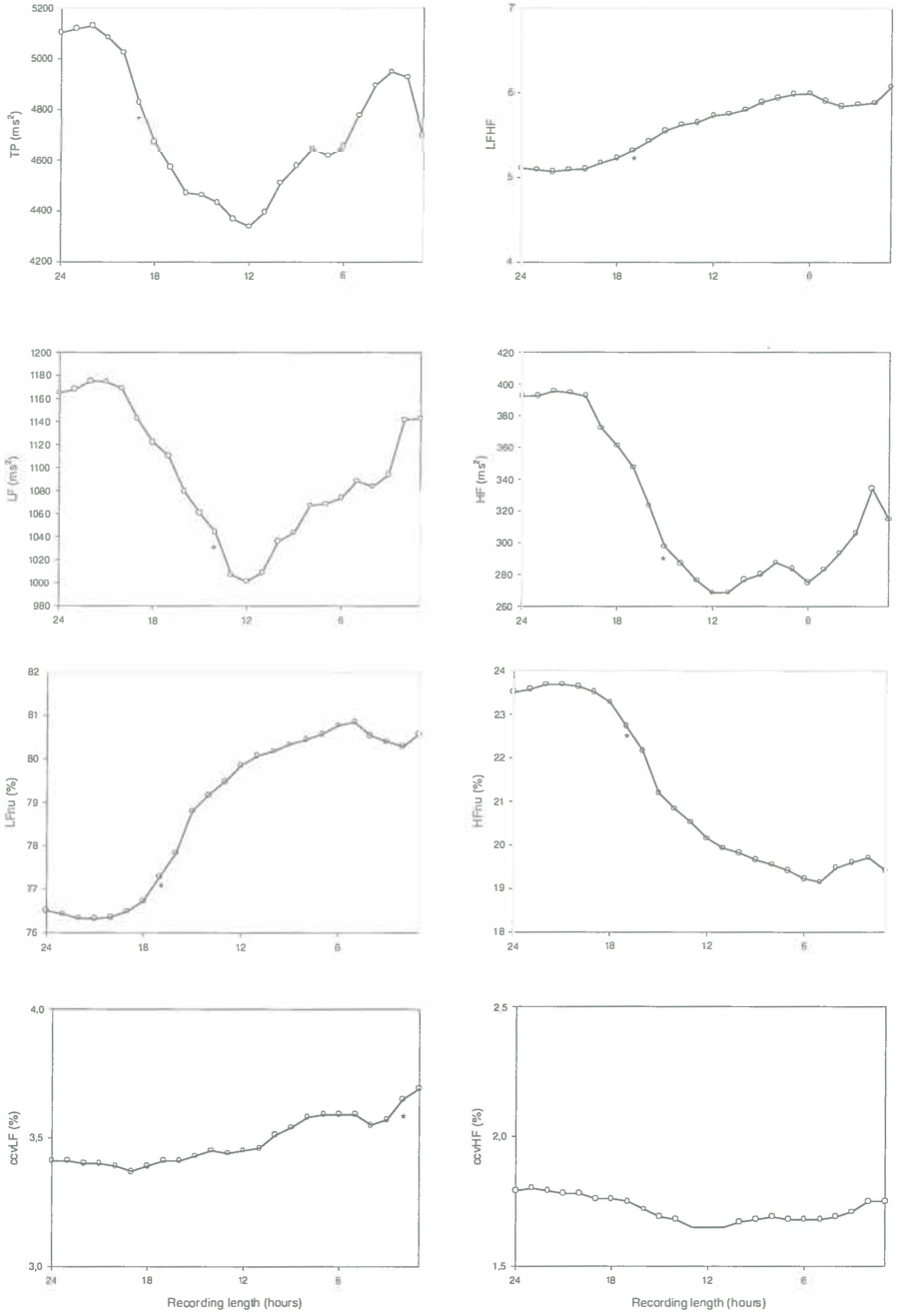


*Figure 33. Influence of recording duration on frequency-domain variables. The * marks the first point where a decrease in recording leads to a significant change in the HRV variable .*

In conclusion, recording duration influences the outcome of HRV analysis for both time- and frequency-domain analysis. This is explained by the fact that hook-up times of ambulatory monitoring recordings are not distributed randomly. Although AVGNN is significantly changed after removal of only 1 hour of the recording, of this change can hardly be considered clinically relevant because of its small magnitude. In general, frequency domain variables are more stable than time-domain variables. In fact, in a normal population, all time-domain HRV variables differ significantly when a recording duration of 18 hours (advised as a minimum by the guidelines) is used in stead of 24 hours. In general recording duration-related changes are slightly higher in patients than in normal subjects. In agreement with the guidelines, we observed that in frequency-domain analysis only TP changes significantly before the 18-hour limit. ccvLF does not change until recording duration is reduced to two hours, while ccvHF does not change significantly at all. If recordings of different duration are compared, using the shortest duration as the standard for such comparison should be considered. For both time- and frequency-domain analysis, recordings with a duration less than 20 hours should not be used.

7.
EFFECTS OF BREATHING

As explained in chapter 1 and 2.4.6, breathing has a profound effect on high-frequency oscillation of the heart rhythm. This is often referred to as respiratory sinus arrhythmia. Metronome breathing is often advocated to assess cardiac vagal control more accurately compared to free breathing. However, the additional value of metronome breathing over spontaneous breathing has never been established. To assess the effect of 0.25Hz metronome breathing on HRV analysis we studied 12 healthy male subjects under stable conditions using pharmacological autonomic blockade[79].

The study was performed in 12 healthy male subjects (mean age 33 years ± 6). The subjects had no history of any major disease. Physical examination, exercise testing, echocardiography and 24-hour ambulatory monitoring revealed no abnormalities. The study was approved by the Institutional Review Board. All subjects gave their written informed consent. During the actual protocol the ECG was recorded using a four-channel Marquette series 8500 recorder. This recorder utilizes a 32 Hz time track to compensate possible tape speed irregularities. At playback the ECG is sampled at 128 Hz real-time. During all stages of the protocol the ECG was directly monitored using a Hewlett Packard 7803B scope. A flow diagram of the study is shown in Figure 34. A needle was inserted into the antecubital vein at the very beginning of the study. No activities were performed during the first 25 minutes in order to exclude a possible effect of needle insertion[227]. After baseline recordings a bolus injection of 0.2 mg/kg propranolol was administered to block sympathetic activity. Twenty-four minutes of recording were started when heart rate was stable. Total autonomic blockade was achieved by adding 0.02 mg/kg methyl-atropine after which a final recording was made, again starting when the heart rate was stable. All three recorded episodes were divided into 12 minutes of spontaneous breathing and 12 minutes of metronome breathing. During the entire procedure the subjects were in a supine position, instructed not to speak and watching a wildlife video movie to ensure stable conditions.

Frequency-domain analysis was performed over 5-minute segments using Discrete Fourier Transformation [58, 176, 178, 179, 203]. From the 12 minutes that were available per breathing stage, two 5-minute segments were analysed. The results of these 2 segments were averaged. All data segments contained less than 1% non-sinus intervals (noise or ectopic data). These non sinus intervals were substituted using linear interpolation. Substitution was based on time, not number of beats[83]. VCC (vagal cardiac control: AVGNN during ß blockade - AVGNN during total blockade) was used as a marker of vagal activity since it is the change in AVGNN due to vagal blockade[85]. All HRV variables were computed in accordance with the guidelines for HRV analysis as given by the task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology[2]. For statistical analysis SPSS version 6.0 was used. Students-t test and Pearson's correlation tests were used to analyse the data. For non-normally distributed variables, Wilcoxon's matched pairs

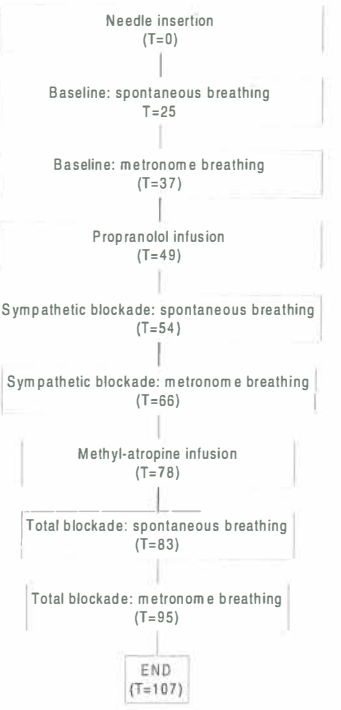


Figure 34. Flow chart of the protocol used to access effects of metronome breathing. The time scale (T) is presented in minutes.

signed ranks test and Spearman's rank correlation coefficients were computed. Results are represented as mean ± standard error of the mean (SEM).

In Table 22 the mean baseline values of the HRV variables during both breathing stages are shown. The mean NN-interval was not influenced by metronome breathing and HFnu showed an increase. On average the values of most HRV variables were lower during metronome breathing compared to spontaneous breathing.

Variable	spontaneous breathing	metronome breathing	p value
AVGNN	1032 ± 39	1022 ± 41	Ns
SD	66.0 ± 10.6	55.0 ± 7.5	0.010
rMSSD	59.7 ± 11.6	48.1 ± 7.3	0.039
sqrt(TP)	55.5 ± 10.2	41.8 ± 5.1	0.032
sqrt(LF)	41.4 ± 7.6	28.7 ± 3.4	0.025
sqrt(HF)	36.4 ± 7.0	29.8 ± 4.1	Ns
LFHF	1.59 ± 0.31	1.09 ± 0.15	0.049
LFnu	52.2 ± 3.5	44.9 ± 3.1	0.019
HFnu	42.1 ± 4.0	49.1 ± 3.4	0.019
ccvLF	4.0 ± 0.63	2.8 ± 0.31	0.013
ccvHF	3.5 ± 0.56	2.9 ± 0.32	0.047

Table 22. Values of baseline HRV variables during spontaneous breathing vs. metronome breathing.

With the exception of LFnu, all baseline HRV variables during spontaneous breathing and metronome breathing, were correlated though the extent varied. Correlations are shown in Table 23.

Variable	correlation coefficient (r)	p-value
AVGNN	0.97	< 0.001
SD	0.98	< 0.001
rMSSD	0.97	< 0.001
sqrt(TP)	0.95	< 0.001
sqrt(LF)	0.88	< 0.001
sqrt(HF)	0.97	< 0.001
LFHF	0.63	0.028
LFnu	0.45	Ns
HFnu	0.69	0.014
ccvLF	0.87	< 0.001
ccvHF	0.97	< 0.001

Table 23. Correlation coefficients between baseline HRV variables during spontaneous breathing and metronome breathing.

variable	spontaneous breathing	metronome breathing	p value
AVGNN	1216 ± 48.4	1223 ± 58.8	Ns
SD	88.9 ± 17.1	72.5 ± 9.6	Ns
rMSSD	92.8 ± 21.2	80.3 ± 13.6	Ns
sqrt(TP)	75.8 ± 18.3	60.4 ± 9.8	Ns
sqrt(LF)	51.8 ± 12.7	33.6 ± 4.6	Ns
sqrt(HF)	54.6 ± 13.5	49.2 ± 9.6	Ns
LFHF	1.07 ± 0.18	0.65 ± 0.13	0.041
LFnu	43.7 ± 3.7	32.7 ± 3.7	0.012
HFnu	48.7 ± 4.0	62.3 ± 4.5	0.010
ccvLF	4.0 ± 0.7	2.7 ± 0.3	Ns
ccvHF	4.2 ± 0.8	3.8 ± 0.5	0.047

Table 24. Values of HRV variables during sympathetic blockade: spontaneous breathing vs. metronome breathing.

variable	correlation coefficient (r)	p-value
AVGNN	0.96	< 0.001
SD	0.90	< 0.001
rMSSD	0.86	< 0.001
sqrt(TP)	0.97	< 0.001
sqrt(LF)	0.86	< 0.001
sqrt(HF)	0.97	< 0.001
LFHF	0.45	Ns
LFnu	0.37	Ns
HFnu	0.55	Ns
ccvHF	0.80	0.002
ccvLF	0.84	0.001

Table 25. Correlation coefficients of HRV variables between spontaneous breathing and metronome breathing during sympathetic blockade.

HF variables showed stronger correlations than LF variables (absolute, normalized units and CCV). Comparing the same breathing stages during β-blockade also shows a decrease during metronome breathing again except for HFnu (Table 24).

During both baseline and sympathetic blockade, the absolute values of HRV variables during metronome breathing were lower than during spontaneous breathing. Although significant differences are present, strong correlations existed also during sympathetic blockade as shown in Table 25.

Examples of the baseline HRV spectrum during spontaneous breathing and metronome breathing are shown in Figure 35. During spontaneous breathing, the power in the HF band is "scattered" over the entire 0.15 - 0.40 Hz range. During metronome breathing power is predominantly concentrated around the breathing frequency (0.25 Hz). This results in a moderate improvement of the correlation coefficients during metronome breathing compared to

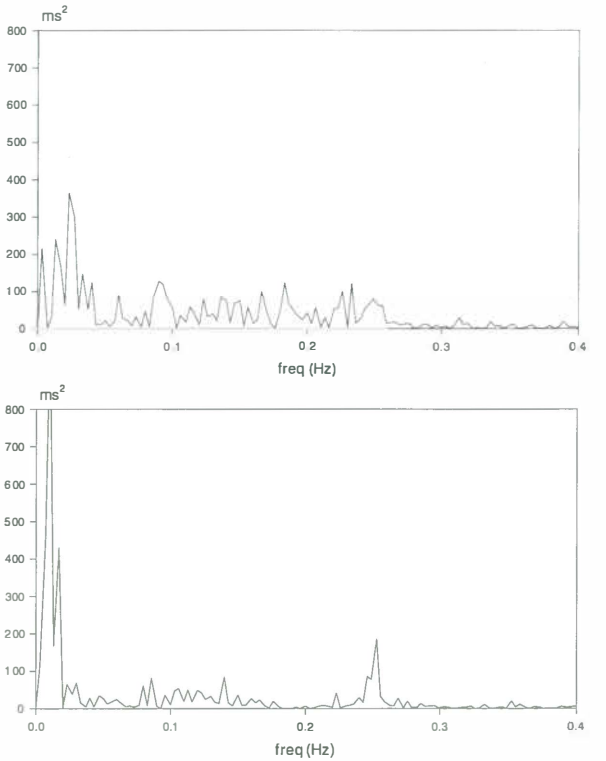


Figure 35. Heart rate spectrum during spontaneous breathing (upper panel) and during metronome breathing (lower panel). During metronome breathing HF power is concentrated more around the breathing frequency of 0.25 Hz.

	spontaneous breathing		metronome breathing	
	r	p	r	p
		at baseline		
sqrt(HF)	0.74	0.006	0.79	0.002
HFnu	0.41	Ns	0.70	0.012
		during sympathetic blockade		
sqrt(HF)	0.72	0.009	0.76	0.004
HFnu	0.48	Ns	0.59	< 0.05

Table 26. Correlation coefficients between HRV variables and VCC at baseline and during sympathetic β-blockade.

spontaneous breathing (Table 26). The correlation coefficient between VCC during spontaneous breathing and VCC during metronome breathing was 0.98 ($p < 0.001$).

Conclusion: Practical experience shows that metronome breathing can only be maintained for relatively short periods of time. Therefore, metronome breathing is only applicable during "laboratory conditions". In this setting, metronome breathing has some advantages over spontaneous breathing in HRV measurements. For example metronome breathing of 0.25 Hz will prevent slow breathing patterns from interfering with the LF frequencies. However, using commonly accepted frequency ranges ensures the inclusion of all breathing-related modulations, in the high frequency range usually 0.15-0.40 Hz. The remaining differences are limited and may therefore be neglected when assessing HRV using routine clinical ECG recordings. Furthermore, metronome breathing is often experienced as unpleasant and even stress-inducing. As such it may even be considered contra-indicated when assessing autonomic control.

8.
HEART RATE DEPENDENT CHANGES IN FREQUENCY-DOMAIN ANALYSIS

In HRV analysis, resampling techniques like linear interpolation are frequently used in combination with FFT for frequency-domain analysis. It has been reported that resampling techniques may act as a low pass filter[106]. Therefore the outcome of HRV analysis may vary depending on heart rate itself.

In order to investigate the influence of heart rate on the outcome of frequency-domain analysis simulated as well as real life ECG was used. In a simulation we created 5-minute episodes of an artificial test signal by modulating a fixed interval with a sinus of a known frequency. Test signals with an average interval of 600, 800 and 1000 ms were modulated from 0 to 0.4 Hz in steps of 0.05 Hz. The effect of non-stationarity was tested by introducing a trend in the artificially created time series. These 300 second data segments were analysed using DFT [58, 176, 178, 179, 203]) and a 1024 point FFT (sample distance: 300/1024 = 293 ms, sample frequency: 1000/293 = 3.4 Hz). Finally a test with a true signal was performed. A one hour and 5-minutes ambulatory ECG registration was recorded from a young healthy female, using a three-channel Marquette series 8500 recorder. This recorder utilizes a 32 Hz time track to compensate for tape speed irregularities. The ECG was visually checked for ectopic beats and noise after which the RR-interval series was transferred to the COHORT system for detailed analysis. During the recording, the subject was submitted to a mental and physical stress test respectively. The resampling techniques used with FFT were:

- linear interpolation
- cubic spline interpolation
- the "Berger" algorithm [16]

All these techniques have frequently been used in clinical practice. For the FFT techniques a Hanning window was applied to minimize spectral leakage. The artificially created RR-interval series were compared to the outcome of the frequency-domain analysis according to the theorem of Parseval (Equation 1). This theorem states that the power of a signal in time-domain equals the power of a signal in frequency domain.

The total power reproduced by frequency-domain analysis as a percentage of the actual power (variance of the RR-interval series) is shown in Figure 36 to Figure 38.

TEST SIGNALS:

FFT and linear interpolation:

An example of resampling using linear interpolation is shown in Figure 22. Using FFT and linear interpolation returned power decreased not only with increasing modulating frequencies but also with increasing average RR-interval. When an average RR-interval of 1000 ms was modulated with a 0.4 Hz sinusoidal modulation only 40% of the actual power was returned (Figure 36).

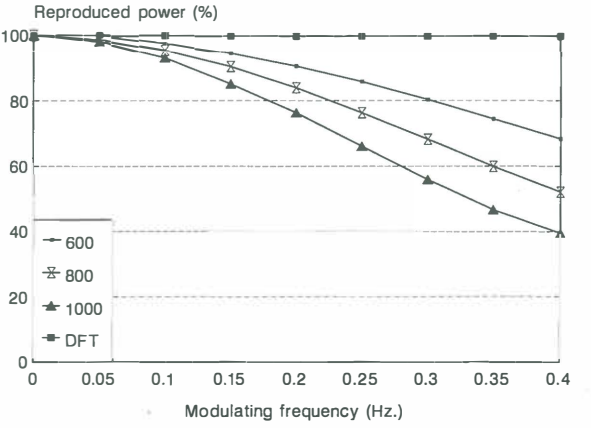


Figure 36. FFT using linear interpolation. Less power is reproduced at lower average heart rates. This is especially true for high modulating frequencies. The result of DFT is shown as solid squares.

FFT and cubic spline interpolation:

Cubic spline interpolation fits a third order polynoma through the original time series. Using cubic spline interpolation, the frequency-dependent changes are considerably smaller compared to linear interpolation. Nevertheless in the worst case (1000 ms average RR-interval, 0.4 Hz modulation) up to 1/3 of the actual power is lost (Figure 37)

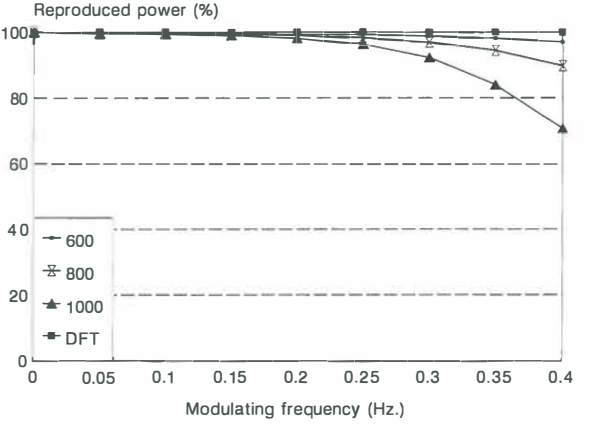


Figure 37. FFT and cubic spline interpolation. The loss of power at low average heart rates is less compared to FFT and linear interpolation but can still clearly be observed. The result of DFT is shown as large squares.

The Berger algorithm:

Berger et al. described an algorithm to interpolate ECG, that incorporates the contribution of successive RR intervals within a local window[16]. The heart-rate dependent changes are virtually absent using the Berger algorithm for resampling, however the low pass filter effect still accounts for a power reproduction of only 65% when a 0.4 Hz modulation was applied on a 1000 ms average RR-interval. (Figure 38).

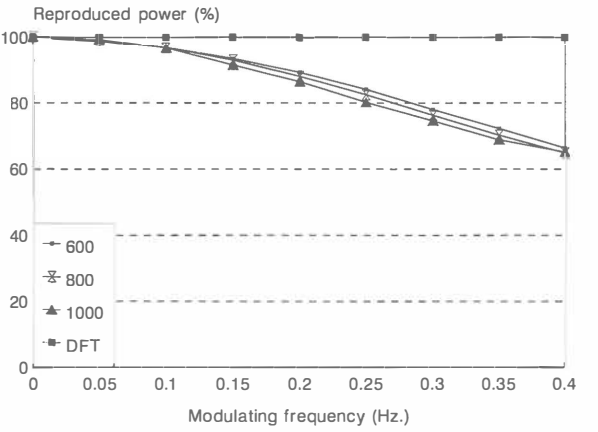


Figure 38. FFT and the Berger algorithm. The loss of power as a consequence of lower average heart rates is a linear process. Again the effect is strongest at the highest modulating frequencies. The result of DFT is shown as large squares.

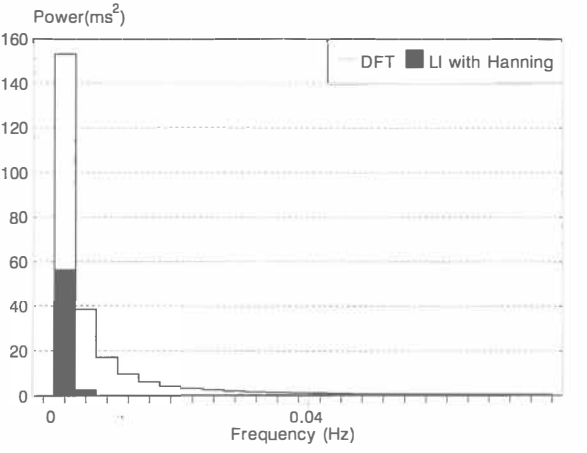


Figure 39. Non-stationary signal in DFT and FFT_linear interpolation (LI). The consequence of the Hanning window is a considerable loss of power, compared to the actual power of the signal. Although more smearing is present, power is better reproduced by DFT.

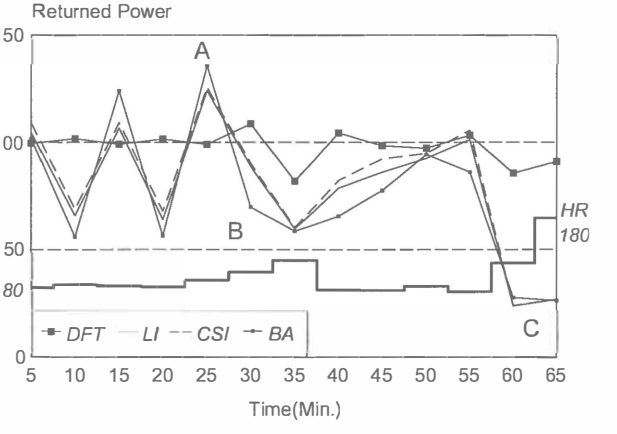


Figure 40 Percentage of returned total power (DFT = Discrete Fourier Transformation, LI = FFT using Linear interpolation, CS = FFT using cubic spline interpolation, BA the FFT using the Berger algorithm)

DFT: The DFT results shown in each figure represent the artificial test signal with an average RR-interval of 1000 ms. For all methods using FFT and resampling, this was the worst case scenario, the situation in which most loss of power was observed.

An important difference between the test-signals described before and real ECG data is the fact that the average frequency of real ECG data may vary. To test the effect of non-stationary signals an average RR-interval of 1000 ms was modulated with a 0.00010 Hz sinusoidal modulation. The result of this modulation (with an amplitude of 300 ms) is a time series that increases from 1000 to 1055 ms in 300 sec. Figure 39 shows the result of frequency-domain analysis by DFT and FFT using linear interpolation. Cubic spline interpolation and the Berger algorithm gave almost identical results. DFT accurately reproduces the total power, however a certain amount of spectral power is "smeared" to the surrounding frequencies. FFT & linear interpolation cause a considerable loss of power; only 23.7% of the actual power is returned. When the Hanning window was removed, the results of FFT using resampling were similar to that of DFT. As can be seen in Figure 39, the influence of the smearing is diminished at frequencies above 0.04 Hz. Therefore, it is likely that non-stationarity will have little effect when using DFT and the limits usually applied for LF and HF computations.

REAL LIFE ECG:

Finally, to study heart rate-dependent changes in a "real life" signal we selected an ECG recording containing changes in heart rate due to mental as well as physical exercise. The recording consisted of a 20 minute baseline after which the first increase in heart rate represents two mental stress tests (arithmetic tests) while after a second baseline period of 20 minutes a physical exercise test was performed. Figure 40 shows the returned total power again as a percentage of the power in time-domain. All thirteen 5-minute episodes were of high quality (maximum percentage noise & ectopic beats in 1 segment: 1.67%). The noise segments were corrected by means of linear interpolation. It is clear that the total power computed by DFT is closer to the power in time-domain when compared to the power returned by FFT using the various resampling techniques. The average percentage of returned total power for DFT was 97.8% , for linear interpolation 78.8%, cubic spline interpolation 81.4% and the Berger algorithm 75.5%. Three special points of interest are marked in Figure 40 (A,B and C). The first point (A) represents the first mental stress test at which spectral power is clearly overestimated by FFT using the resampling techniques. The explanation for this fact can be seen in Figure 41.

Figure 41. Heart rate at point A (see Figure 40).

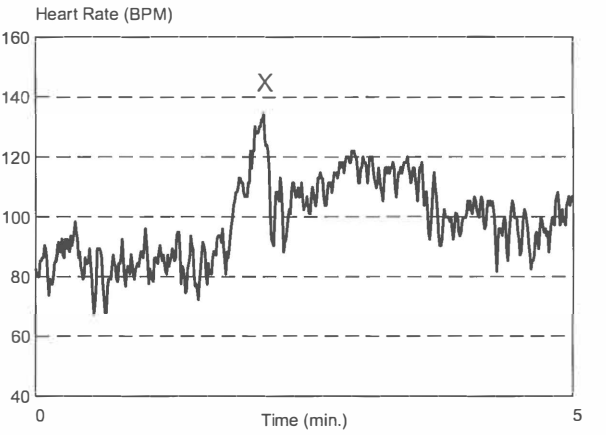
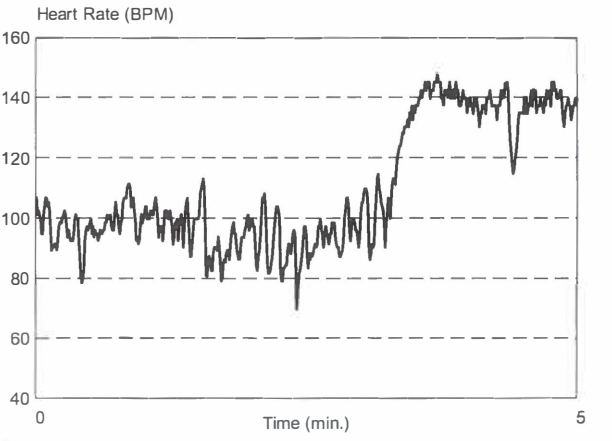


Figure 42. Heart rate at point B (see Figure 40)



A relatively short increase in heart rate in the middle of the episode (marked X) is fully amplified by the Hanning window, while influence of the rest of the signal is relatively reduced. In Figure 40, at points B (Figure 42) and C (onset of mental stress test 2 and onset of the physical exercise test) 10 minutes of non stationary signal cause underestimation of total power by all techniques. Again however it is clear that DFT more closely represents true power in these episodes than FFT using the various resampling techniques.

Computational Speed:

Taking into account the fact that speed was the historical significance of FFT, we determined the average processing time per technique. The extra steps needed for FFT (resampling) can account for up to 50% of the computational time. In fact DFT proved to be the fastest method (14.6 sec.) in this specific setting.

Frequency-domain analysis using FFT and resampling cause considerable loss of power. For linear interpolation and cubic spline interpolation this phenomenon is dependent on hart rate and modulating frequency. The Berger algorithm is also dependent on modulating frequency, however, the average heart rate has no effect. The difference between linear interpolation, cubic spline interpolation and the Berger algorithm may be explained by the fact that the first two resampling techniques are inter beat interval spectra and the last one is a heart rate spectrum[58]. DFT continues to accurately reproduce spectral power no matter what average RR-interval was used. The explanation for the fact that non-stationary signals cause more smearing with DFT compared to FFT and resampling, is the Hanning window, which that is only applied in the FFT techniques. The analysis of consecutive (ECG) data segments, a common phenomenon in HRV analysis, inevitably leads to inclusion of non-stationary sections. The position of the non-stationarity within a segment in relation to a window plays an important role in the computation of spectral power. In general it is obvious that the selection of an analysis method will influence the outcome of the analysis. Methods for frequency-domain analysis like DFT, which do not require resampling techniques are therefore preferable, especially since computational speed is no longer a problem using modern computers.

9.
HRV IN ATRIAL FIBRILLATION

HRV is analysed using sinus rhythm and special care is taken to exclude NSI, as discussed chapter 3.2. However, HRV may also be of interest in non-sinus rhythm such as atrial fibrillation especially given the important role of the autonomic nervous system in causing atrial fibrillation. In addition, atrial fibrillation itself produces autonomic activation. Atrial fibrillation is a common rhythm disturbance, especially in elderly people. In the majority of cases structural heart disease is demonstrable, notably ischaemic heart disease and hypertensive heart disease. Also, heart failure is an important precursor condition [113,126]. However, in a sizeable portion of patients no structural disorders are present ("lone" arrhythmia)[33]. According to the prevailing concept, the mechanism underlying atrial fibrillation is "multiple wavelet re-entry" [7,166-168] implying that multiple (>4) wavelets continuously traverse the atria in an ever-changing (random) fashion re-exciting atrial tissue once excitability is just restored. The clinical hallmark of atrial fibrillation is an irregular (random) heart beat, which is due to random input into the atrioventricular (AV) node causing varying degrees of concealed conduction within the AV node [73,173]. According to the duration of the arrhythmia two forms are distinguished: paroxysmal atrial fibrillation and chronic atrial fibrillation. Based on clinical and electrophysiological considerations, atrial fibrillation terminating spontaneously within 24-48 hours is designated paroxysmal atrial fibrillation, whereas longer attacks constitute chronic atrial fibrillation.

Paroxysmal atrial fibrillation. Several lines of evidence suggest that the autonomic nervous system is of importance in the genesis of paroxysmal atrial fibrillation, particularly in case of lone arrhythmia. Thus, the clinical history in some patients suggests that increased vagal activity precipitates the arrhythmia [50,206]. Attacks typically occur at rest, after meals, or during the night. In fact, patients often deliberately start exercising to terminate the attacks. In these cases electrocardiographic findings often support a vagal genesis since onset of atrial fibrillation is preceded by progressive slowing of heart rate. Electrophysiologic considerations support the concept of "vagal" atrial fibrillation. Under experimental conditions, vagal stimulation has been shown to facilitate atrial fibrillation by shortening the atrial refractory period (and hence the wavelength of atrial impulses) and by increasing dispersion of refractoriness [8,113]. In fact, vagal stimulation, either by electrical stimulation of the vagus nerve or topical administration of vagomimetic agents, is an established method to induce experimental atrial fibrillation [181]. Finally, clinical practice supports the concept of vagal atrial fibrillation in that beta-blockers and digoxin are usually of little avail and may actually aggravate the arrhythmia, whereas patients often respond favourably to vagolytic agents such as disopyramide. Conversely, the clinical picture in other patients suggests a sympathetic origin of atrial fibrillation; again the clinical history and electrocardiographic findings are crucial in this connection [50,206], but also electrophysiologic data support the concept of "sympathetic" atrial fibrillation [75,215].

The data in the literature on vagal and sympathetic atrial fibrillation are largely anecdotal and systematic research is needed. We hypothesized that analysis of HRV might be helpful in this respect. It was argued that by analysing HRV during sinus rhythm preceding onset of atrial fibrillation one might demonstrate specific autonomic forms, notably a vagal mechanism. Chapter 9.1 deals with this issue by showing the value of HRV in establishing the definitive diagnosis and guiding therapy in a case of presumed vagal atrial fibrillation. The case has already been published in a summarized version; herein an extended version will be given.

Chronic atrial fibrillation. Despite the fact that the clinical hallmark of atrial fibrillation is an irregular heart beat, clinicians are well aware of the fact that mean heart rate in individual patients with atrial fibrillation varies depending on the level of activity, comparable to variations in heart rate in subjects with sinus rhythm. Heart rate is lowest at night and at rest, whereas highest rates occur during the day and physical activity [11, 86, 242]. These simple clinical observations suggest an important role for the autonomic nervous system. This is supported by the effects of certain pharmacologic agents on heart rate in atrial fibrillation. Thus, vagolytic agents like atropine increase heart rate [76, 100] whereas beta-blockers lower heart rate. In fact, the latter agents are widely used in clinical practice to control rate. Finally, autonomic manoeuvres, for instance carotid sinus activation, exert a direct effect on heart rate [218].

The obvious clinical effect of the autonomic nervous system on the heart rate in atrial fibrillation (ventricular rhythm), is primarily attributed to the effect on the AV node. According to the prevailing concept, the AV node is the principal determinant of ventricular rhythm in atrial fibrillation, the refractoriness of the node restricting AV transmission of the atrial fibrillatory impulses [24, 232]. A direct effect of the autonomic nervous system on input into the AV node probably also plays a role in atrial fibrillation[244]; in particular vagal activation through shortening atrial refractoriness increases fibrillatory activity and hence input into the AV node. This in turn will lead to enhanced concealed conduction within the AV node causing depressed conductivity and thus slower ventricular rhythm. Consequently, vagal effects on concealed conduction and fibrillatory rate act in concert. Sympathetic activity increases ventricular rate, probably largely due to shortening of the refractoriness of the AV node[243, 245].

Based on these considerations, we argued that analysis of HRV might be a meaningful method for assessment of vagal activity in patients with atrial fibrillation, analogous to analysis of HRV in subjects with sinus rhythm. In other words, we considered the possibility that HRV in atrial fibrillation reflects vagal activity despite the complete irregularity (randomness) of ventricular rhythm. This possibility was investigated in chapter 9.2. It was shown that HRV in patients with atrial fibrillation relates to vagal activity.

Chapter 9.1 deals with a typical example of paroxysmal vagal atrial fibrillation[246], while in chapter 9.2 autonomic influences are studied in patients with chronic atrial fibrillation.

9.1 PAROXYSMAL ATRIAL FIBRILLATION

In a subset of patients with paroxysmal lone atrial fibrillation, augmented activity of the autonomic nervous system is deemed responsible for the arrhythmia. We report the case of a 46-year-old male with clinical features suggestive of "vagal" atrial fibrillation, who was successfully treated with disopyramide. Analysis of heart rate variability confirmed both the clinical suspicion on vagally-mediated arrhythmia and the vagolytic effect of disopyramide.

Introduction

In a subset of patients with paroxysmal atrial fibrillation no structural heart disease is demonstrable ("lone" atrial fibrillation). Augmented activity of the vagal nervous system is responsible for the arrhythmia in some of these patients[49]. Though the prognosis of "vagal" atrial fibrillation is benign, effective control of symptoms (palpitations) is cumbersome. Occasionally, class IA agents may prove beneficial, which is attributed in part to their anticholinergic properties. Here we describe a patient with vagal atrial fibrillation who was successfully treated with disopyramide, a class IA agent with substantial anticholinergic effects[164]. Analysis of heart rate variability was performed to gain more insight into the underlying mechanisms

Case report

A 46-year-old healthy male presented with a 3-year history of episodic palpitations. The frequency of the attacks was at least two to three times per week and the duration ranged from a few minutes to several hours. Typically, the attacks occurred at rest and during sleep. Often an attack could be terminated by physical exercise. Prior medications included digoxin, verapamil, atenolol, pindolol, xamoterol, sotalol (80 mg thrice daily), and flecainide (200 mg twice daily). None of these agents was of any avail. The electrocardiogram was unremarkable, laboratory findings were normal, including electrolytes and thyroid function. Also, echocardiography and bicycle ergometry were normal. In particular, no rhythm disturbances were noted, either during exercise or recovery. Ambulatory 24-hour electrocardiographic (Holter) monitoring showed that the palpitations were due to paroxysmal atrial fibrillation. Three episodes of atrial fibrillation were recorded; two attacks occurred in the evening and one attack occurred during sleep. The duration of the episodes was 46 seconds, 40 minutes and 2 hours, respectively. Detailed analysis of the

Figure 43 Onset of atrial fibrillation during slow preceding sinus rhythm

recordings strengthened the clinical suspicion on vagally-mediated arrhythmia; a slow sinus rate preceded the onset of atrial fibrillation and the ventricular response during atrial fibrillation was slow (90-110 beats/min) (Figure 43)

In order to further clarify the role of the autonomic nervous system, analysis of heart rate variability was performed. The following time-domain and frequency-domain components of heart rate variability were analysed in 2-minute segments before onset of atrial fibrillation; the standard deviation of normal RR-intervals, the percentage of successive normal RR-intervals differing by >50 ms (pNN50), low-frequency (0.04-0.15 Hz) and high-frequency (0.15-0.40 Hz) spectral power, and the ratio of low-frequency to high-frequency power (LFHF ratio). Spectral power was computed using Fourier analysis[5]. High-frequency power and pNN50 are measures of vagal activity, whereas low-frequency power is considered to reflect both sympathetic and vagal activity. Since ectopic activity precludes accurate analysis of heart rate variability, atrial (and ventricular) premature beats were carefully labelled and excluded from the analysis. Results are listed in Table 1. The data were indicative of a progressive increase in vagal tone preceding atrial fibrillation. The patient was then started on disopyramide 250 mg twice daily. This resulted in almost total relief of the arrhythmia; rarely (less then once a month) did the patient still experience palpitations. Increasing the dose to 250 mg three times daily eliminated the remaining attacks. However, side-effects including dryness of mouth and blurring of vision precluded maintaining the patient on this high dose. To investigate the mechanism underlying the beneficial effect of disopyramide heart rate variability was analysed again, comparing a 24-hour Holter recording while the patient was on disopyramide (250 mg twice daily)

with the first recording. (The three episodes of atrial fibrillation were excluded from the analysis.) Results are summarized in Table 2, representing mean values over 24 hours. Findings were suggestive of a substantial vagolytic effect of disopyramide.

Discussion

Analysis of heart rate variability is a new, non-invasive diagnostic tool for assessment of autonomic status. Thus far only few investigators have employed this technique in the setting of paroxysmal atrial fibrillation[49, 55, 165, 210]. Our findings support these preliminary studies; onset of the arrhythmia in our patient with clinical features suggestive of vagally-mediated arrhythmia was also preceded by a progressive increase in heart rate variability measures of vagal activity. Interestingly, like Mitsuno and co-workers[165], who similarly analysed 2-minute segments, we observed that the actual onset of atrial fibrillation was immediately preceded by a "final" surge in vagal tone. In addition, we noted another intriguing phenomenon that was also reported by Mitsuno et al; low-frequency power also increased before onset of atrial fibrillation. (As a result, the ratio of low-frequency to high-frequency power was not significantly affected.) This may have been due merely to the vagal hyperactivity, which also affects low-frequency power. Alternatively, it may reflect sympathetic activation, which would suggest that in "vagal" atrial fibrillation concomitant increases in sympathetic activity, i.e., sympathovagal interactions play a role. It thus appears that analysis of heart rate variability may contribute to our understanding of the pathophysiology of paroxysmal atrial fibrillation and may be a useful diagnostic tool in the work-up of these patients. Furthermore, our findings suggest that it may also guide therapeutic management. Disopyramide was prescribed because of its strong anticholinergic properties. Analysis of heart rate variability confirmed that disopyramide indeed exerted vagolytic effects. Obviously, this does not prove a causal relation between the beneficial effect of the drug in this patient and the effect on the vagal nervous system. Nevertheless, the observation that flecainide and sotalol (class I and III anti-arrhythmic activity, respectively) did not suppress the arrhythmia suggests that the vagolytic effect of disopyramide may have played an important role in this particular case.

9.2 CHRONIC ATRIAL FIBRILLATION

Background

Analysis of HRV has thus far not been applied in patients with atrial fibrillation, probably because of the presumed absence of any form of patterning of the ventricular rhythm, particularly vagally-mediated respiratory arrhythmia.

Minutes before onset	10-8	8-6	6-4	4-2	2-0
meanRR (ms)	916	922	908	937	947
RR SD (ms)	32	37	34	35	40
pNN50 (%)	3.4	3.0	4.8	4.5	7.9
LF power(ms^2)	609	528	725	824	1182
HF power(ms^2)	149	154	169	215	347
LFHF ratio	3.81	4.08	3.51	3.52	3.53

Table 27. Mean values of components of heart rate variability in 5 consecutive 2-minute segments preceding 3 episodes of atrial fibrillation.
HF, high-frequency band (0.15-0.4 Hz); LF, low frequency band (0.04-0.15 Hz); LFHF ratio, ratio of low-frequency to high-frequency power; pNN50, percentage of successive normal RR-intervals differing by >50 ms; RR SD, standard deviation of normal RR-intervals.

	before disopyramide	after disopyramide
mean RR (ms)	854	817
RR SD (ms)	55	44
pNN50 (%)	3.2	1.8
LF power (ms^2)	803	537
HF power (ms^2)	153	102
LFHF ratio	6.16	7.51

Table 28. Overall 24-hour values of components of heart rate variability before and after disopyramide

However, such patterning is theoretically conceivable given the function of the atrioventricular node in atrial fibrillation and its susceptibility to autonomic influences.

Methods and Results

Sixteen patients (mean age 56±4 years) with long-term atrial fibrillation on fixed doses of digoxin or verapamil were studied; 12 healthy men in sinus rhythm were used as control subjects. HRV (standard deviation of RR-intervals (SD), coefficient of variance (CV), the root-mean-square of successive difference (rMSSD), and low-frequency (LF) and high-frequency power (HF)) were analysed during 500 RR-intervals at baseline, after administration of propranolol (0.2 mg/kg IV), and after subsequent administration of methylatropine (.02 mg/kg IV). HRV at baseline and changes in HRV after methylatropine were then related to vagal tone (vagal cardiac control), quantified as the decrease in mean RR after methylatropine. Baseline HRV was higher in the atrial fibrillation group than in the control group; after propranolol, HRV increased in both groups; after methylatropine, HRV neared zero in the control group whereas it returned to baseline values in the atrial fibrillation group. SD, rMSSD, LF and HF at baseline were significantly ($p<.05$) correlated with vagal tone in the control group but also in the atrial fibrillation group (correlation coefficients .60, .61, .57, and .64, respectively). Even stronger correlations were

observed between changes in these parameters after methylatropine and vagal tone, particularly in the atrial fibrillation group (correlations coefficients .89, .87, .72, .90, respectively).

Conclusion:
This study shows that HRV is related to vagal tone in patients with atrial fibrillation.

In recent years, analysis of HRV has emerged as a valuable non-invasive tool for assessment of autonomic status. It has become increasingly clear that various cardiovascular disease states are associated with typical changes in HRV. For instance, heart failure is characterized by HRV changes indicative of sympathetic activation as well as vagal withdrawal[27, 44, 211]. The same holds true for coronary artery disease[86] and valvular heart disease[222]. Moreover, HRV has a prognostic value[122], and pharmacologic interventions may improve HRV, in particular the vagal components[38, 66, 235]. The above findings, however, pertain only to patients with sinus rhythm. A paucity of data exists as to HRV in patients with atrial fibrillation. In fact, atrial fibrillation is generally considered an exclusion criterion for analysis of HRV. Presumably, the apparent total irregularity of ventricular rhythm in atrial fibrillation has daunted most investigators. Yet, atrial fibrillation is very common particularly in patients with heart failure, and also in patients with coronary artery disease and valvular heart disease[72]. In a single study, the prognostic value of several commonly used HRV variables was analysed in patients with valvular disease and atrial fibrillation; interestingly, a decreased HRV was associated with an adverse clinical course[221]. However, basic methodologic and mechanistic aspects were not addressed. In particular, it is unknown whether at all, let alone to what extent, the established time- and frequency-domain HRV variables reflect autonomic status in patients with atrial fibrillation. In the present study we addressed this issue, focusing on the vagal limb of the autonomic nervous system. Sequential pharmacological autonomic blockade was performed in 16 patients with atrial fibrillation by administering first propranolol and then methylatropine; after thus eliminating confounding sympathetic effects, vagal tone and the relation of vagal tone with HRV could be determined. Twelve healthy men served as control subjects.

Methods

Patients
Male or female patients above 18 years hospitalized for elective electrical cardioversion of chronic atrial fibrillation were eligible for the study. Patients with suspected or documented atrioventricular conduction disturbances were excluded, as well as patients with contra-indications for administration of propranolol and atropine. Healthy men were used as control subjects; exercise

testing and echocardiography were performed to exclude cardiovascular disease in these subjects. The study was approved by the institutional review board, and written informed consent was obtained from each participant before entry into the study.

Experimental Protocol

The patients were in the postabsorptive, unsedated state, and lying supine. During the experiment, ventricular rhythm was continuously recorded, with a Marquette Holter recorder (Series 8500). Three electrocardiographic leads were used: modified leads V1, V5, and aVF. After recording of baseline rhythm for 15 minutes, sequential pharmacologic autonomic blockade was performed; a bolus of propranolol (0.2 mg/kg intravenously) was administered to achieve complete β-blockade, and, after 15 minutes, a bolus of methylatropine (0.02 mg/kg intravenously) was added for complete vagal blockade, thus also obtaining complete autonomic blockade[108]. The administration of propranolol and methylatropine was unblinded. After the experimental protocol patients underwent electrical cardioversion, as described previously.[247] Recordings and autonomic blockade were performed in a similar fashion in the control subjects.

Data Analysis

The recordings were processed by an experienced analyst using a Marquette Laser Holter system (Series 8000 XP). Thereafter, three episodes of atrial fibrillation (baseline, after propranolol, and after methylatropine), each containing 500 ventricular intervals, were transferred to a post-processor, developed at our institute[80]. To ensure stable conditions, particularly after drug administration, in each instance the last 500 intervals near the end of each 15-minute recording period were selected. To verify stability, mean heart rate during the first 50 intervals and the last 50 intervals during each period were compared; a mean difference <5% was considered acceptable. HRV analysis was then performed as described previously,[38, 66, 80] and in accordance with the recommendations from the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology[2]. Discrete Fourier transformation was used for the analysis of the frequency (spectral) domain variables. Inherent to the purpose of the study, we could not use normal-to-normal (NN) intervals in the atrial fibrillation group; instead, ventricular-to-ventricular (RR) intervals were used. Furthermore, since changes in heart rate per se, such as occur after administration of propranolol and methylatropine, may affect HRV, at least during sinus rhythm, 2 additional variables were calculated: 1) the coefficient of variance (CV), defined as the standard deviation of RR-intervals/mean RR, and 2) the coefficient of component variance (CCV), defined as the square root of power/mean RR.[85, 86] The time- and frequency-domain variables thus studied are listed in Table 29. HRV in the controls was analysed in the same way. Obviously, NN-intervals could be used in the control subjects.

Vagal tone was assessed with the use of a previously described method[67, 85, 114]. According to this method, vagal tone, referred to as vagal cardiac control (VCC), can be quantified as the cardiac response to additional vagal blockade in the setting of ß-blockade, that is, after elimination of sympathetic effects. VCC was thus calculated as mean RR after propranolol minus mean RR after methylatropine. Finally, we sought to account for the effect of digoxin on HRV because it was argued that the established vagomimetic effect of digoxin in atrial fibrillation[73, 162] constituted a possible confounding factor. Hence, the above analyses were also performed comparing the patients with and those without digoxin.

Statistical Analysis

Data are given as mean ± 1 SEM, unless indicated otherwise; medians with range are given in case of non-normally distributed values. Pearson's test and Spearman's rank correlation test were used to calculate correlation coefficients between HRV variables at baseline and VCC. Similarly, correlations between changes in HRV variables after administration of methylatropine and VCC were calculated. Findings in patients with and without digoxin were compared using ANOVA. Statistical analyses were conducted with SPSS-PC, version 5.01 (SPSS Inc.); P-values $<.05$ were considered to be significant.

Results

Patients

Sixteen patients were included in the study. Clinical characteristics are presented in Table 30. Thirteen patients used digoxin, verapamil, or a combination, for control of the ventricular rate. Three patients used no such drugs. There were no significant differences in clinical characteristics between the patients with and those without digoxin. The control group consisted of 12 men (mean age 33 ± 2 years). Besides transient mild blurring of vision and dryness of mouth in some subjects, administration of propranolol and methylatropine was uneventful both in patients and control subjects. Cardioversion of atrial fibrillation to sinus rhythm was achieved in 13 patients. All these patients had a PR-interval $\leq .22$ second. All recordings were technically adequate ($<1\%$ noise or ventricular ectopic beats) and stationary.

Heart Rate Variability

Effect of Autonomic blockade on HRV. The results of the sequential administration of propranolol and methylatropine on heart rate and the time- and frequency-domain HRV variables are shown in Figure 44 and Figure 45. Baseline mean RR was short compared with baseline mean NN. Nevertheless, mean RR and mean NN increased comparably after propranolol. In contrast, the response to methylatropine differed; although both mean NN and mean

RR shortened after methylatropine was added, the effect on mean NN was more marked. Whereas mean RR returned to baseline, mean NN reached a value well below baseline. Baseline HRV variables, both time- and frequency domain, were high in the atrial fibrillation group compared with the control group. Increases in HRV variables were observed after propranolol in both groups, although the extent varied. After addition of methylatropine, HRV variables decreased again; however, values in the control group virtually neared zero whereas values in the atrial fibrillation group returned to near baseline. Representative examples of the power spectra at baseline and after drug administration in a single atrial fibrillation patient and a control subject are shown in Figure 46.

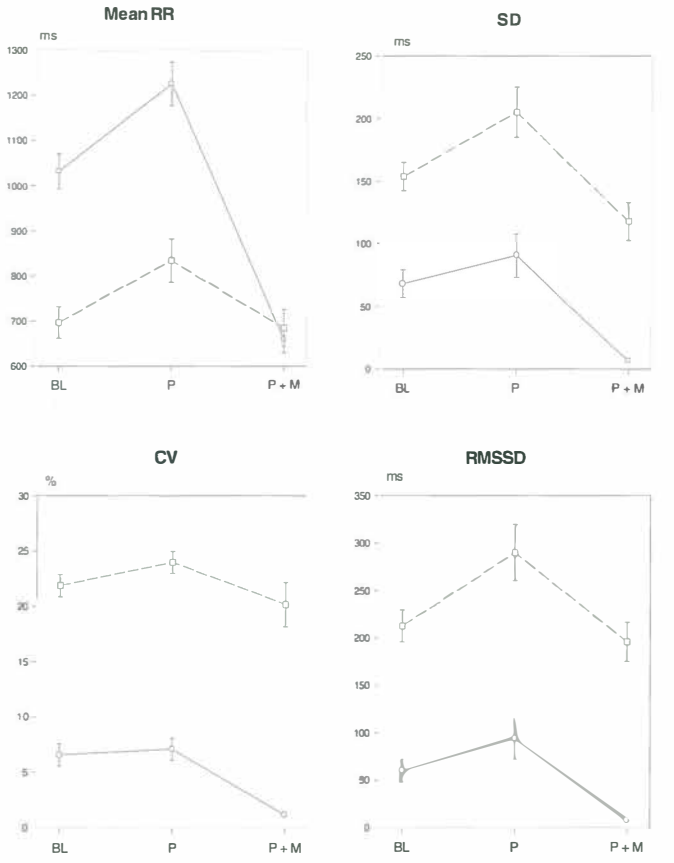


Figure 44. Mean ventricular interval (mean RR) and time-domain variables of heart rate variability (standard deviation of RR-intervals (SD), coefficient of variance (CV), and root-mean-square of successive difference (rMSSD)) at baseline (BL), and the effects of sequential administration of propranolol (P) and methylatropine (M) in the atrial fibrillation group (dotted lines) and the control group (solid lines). Note: error bars in the control group after methylatropine were too small to be depicted with the exception of the mean ventricular interval.

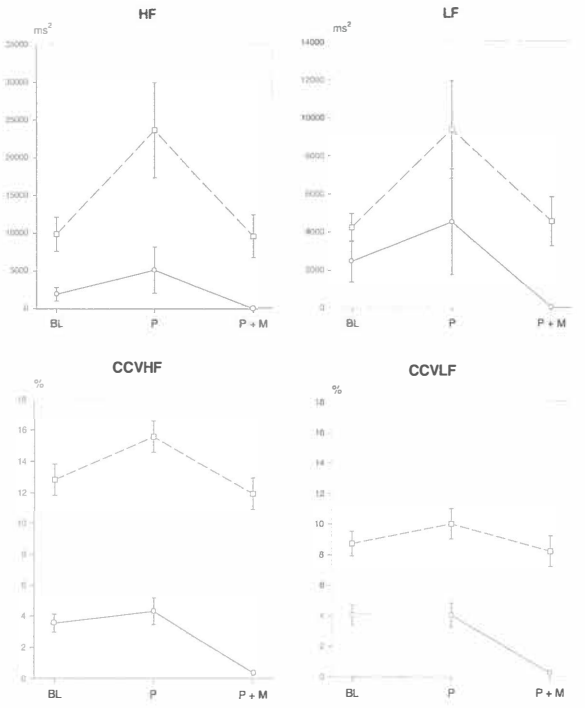


Figure 45. Frequency-domain variables of heart rate variability (high frequency power (HF), low frequency power (LF), and coefficient of component variance of high frequency power (ccvHF) and low frequency power (ccvLF)) at baseline (BL), and the effects of sequential administration of propranolol (P) and methylatropine (M) in the atrial fibrillation group (dotted lines) and the control group (solid lines). Note: error bars in the control group after methylatropine were too small to be depicted.

Correlation Between HRV and Vagal Tone. Correlations between individual variables of HRV at baseline and VCC are given in Table 31. As expected, in the control group significant correlations existed between various variables of HRV and VCC. In fact, all except the CV and CCV of low-frequency power (ccvLF) were correlated with VCC. More importantly, significant correlations between multiple HRV variables and VCC were also found in the atrial fibrillation group. These included the standard deviation of RR-intervals (SD), root-mean-square of successive difference (rMSSD), low-frequency power (LF), and high-frequency power (HF). Correlation coefficients ranged from .57 to .64, with HF showing the strongest correlation. Correlations between changes in individual HRV variables after administration of methylatropine and VCC are given in Table 32. Significant correlations were again observed in the control group for most variables. Also, significant correlations were once more found in the atrial fibrillation group; with the exception of ccvLF, changes after administration of methylatropine in all other HRV variables were correlated with VCC. Correlation coefficients ranged from .72 to .90, HF again showing the strongest correlation. Data in individual patients with respect to HF and ccvHF are shown in Figure 47

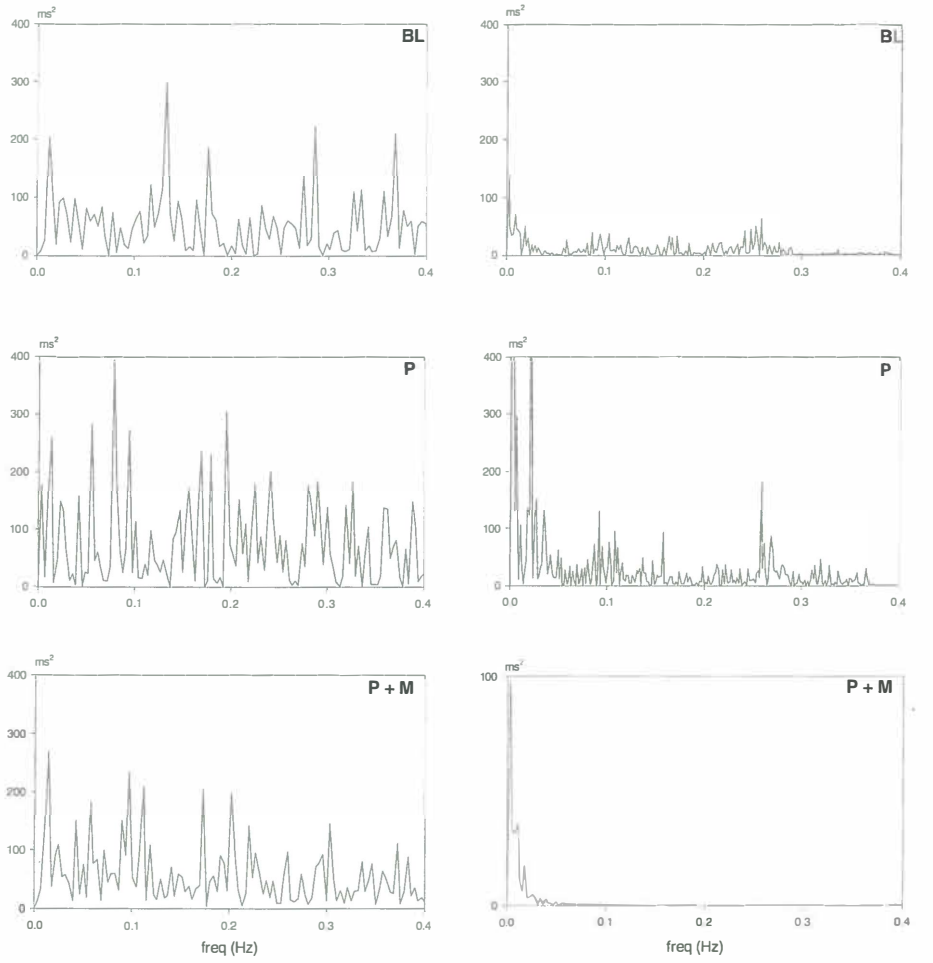


Figure 46. Power spectra at baseline (BL) and after sequential administration of propranolol (P) and methylatropine (M) in a representative patient with atrial fibrillation (left panels) and a control subject (right panels). Note that the scale on the Y-axis is adjusted in the lower right panel. Total spectral power at baseline is clearly higher in the patient with atrial fibrillation. After β-blockade is attained by propranolol, spectral power increases in both the patient and the control subject. Adding methylatropine to propranolol, thereby obtaining complete autonomic blockade, results in almost total diminution of spectral power in the control subject. It also causes a substantial decrease in spectral power in the patient with atrial fibrillation, although spectral power does not near zero as in the control. Importantly, diminution of spectral power includes the high frequency range (.15-.40 Hz), though the change is not confined exclusively to this range.

Role of digoxin. HRV variables at baseline in patients with and those without digoxin did not differ significantly. Also, the responses of these variables to drug administration was comparable. Finally, correlations of the individual HRV variables with VCC did not differ between patients with and those without digoxin. Findings are summarized in Table 33.

Discussion

The principal finding of this study is that HRV in patients with atrial fibrillation is significantly related to vagal tone. Whereas numerous studies have already demonstrated that HRV is a meaningful method to assess vagal tone in patients with sinus rhythm, this study is the first to show that the same holds true for atrial fibrillation patients. It may be surmised that our finding has potentially important clinical implications.

Ventricular Rhythm in Atrial Fibrillation

The clinical hallmark of atrial fibrillation is an irregularly irregular ("random") ventricular rhythm[90]. However, controversy exists as to whether the ventricular rhythm in atrial fibrillation is truly random. Whereas some investigators contend that it is[30], others, using a variety of mathematical techniques, have shown that a certain degree of "patterning" may be present[35, 100, 201, 219]. Still others have investigated respiratory variations of the ventricular rhythm in atrial fibrillation[98, 120, 200, 240]. However, results were conflicting, both within and between the studies; respiratory patterning was an infrequent finding, and in the individuals in whom a certain degree of patterning could be demonstrated, respiration exerted inconsistent effects on ventricular rhythm. Presumably differences in methodology played a role; anyhow, it is noteworthy that in none of the studies spectral analysis was performed, this technique being very well suited for the analysis of respiratory patterning. Moreover, the role of the autonomic nervous system was not addressed. Important questions were thus left unanswered, particularly the possibility of respiratory patterning of the ventricular rhythm due to respiratory fluctuations in autonomic, that is vagal tone. Yet, this is theoretically conceivable, given the electrophysiologic principles governing ventricular rhythm in atrial fibrillation and the importance of autonomic tone. According to the prevailing concept, the principal determinant of ventricular rhythm in atrial fibrillation is the atrioventricular node, refractoriness of the node restricting atrioventricular transmission of the atrial fibrillatory impulses[24, 232]. The irregularity of the ventricular rhythm is considered to be due to the varying degree of penetration of the atrial impulses into the atrioventricular node, thereby causing varying degrees of refractoriness ("concealed conduction")[73, 173]. In addition, although direct electrophysiologic data are scarce, clinical experience provides abundant evidence for a substantial effect of autonomic tone on atrioventricular refractoriness. Thus, the ventricular rhythm

in atrial fibrillation follows a circadian pattern[86], with rates being higher during daytime, for example, during physical exercise, due to vagal withdrawal and sympathetic activity. Lowest rates are being attained during the night due to high vagal tone. A depressant effect of vagal activation on atrioventricular transmission during atrial fibrillation is also apparent from the effect of vagal manoeuvres, for instance, carotid sinus activation[218] as well as from the rise of heart rate after administration of atropine[76, 100].

Present Study

Based on the above premises, we hypothesized that analysis of HRV might be a meaningful method for assessment of vagal tone in patients with atrial fibrillation, analogous to analysis of HRV in subjects with sinus rhythm. The results of the study confirmed our hypothesis. As expected, both time- and frequency-domain variables of HRV at baseline were clearly higher in the patients with atrial fibrillation than in the subjects with sinus rhythm. This reflects the overall higher degree of irregularity of ventricular rhythm in atrial fibrillation. As outlined above, irregularity of the atrial fibrillatory process per se, causing varying degrees of concealed conduction in the atrioventricular node, undoubtedly plays a crucial role in this connection. Yet, the data suggest that "hidden" within the apparent totally irregular rhythm, vagally-mediated respiratory patterning of ventricular rhythm is present. Pertinent to this conclusion is the finding that vagal tone, calculated as VCC, was found to be related to multiple variables of HRV, in particular to HF. Furthermore, changes in HRV variables which occurred after vagal blockade with methylatropine showed even stronger relations with vagal tone. Again, HF, that is, change in HF, showed the strongest relation, the correlation coefficient being as high as .90. To put it differently, it thus appears that a substantial part of the high-frequency (0.15-0.40 Hz) fluctuations of the ventricular rhythm in atrial fibrillation is due to respiratory fluctuations in vagal tone. As such, our study suggests that atrial fibrillation behaves like sinus rhythm. In fact, correlation coefficients at baseline were on the average only slightly lower in the atrial fibrillation group than in the control group with sinus rhythm. At this stage, however, it should be pointed out that HRV does not appear to be an absolute measure of vagal tone in patients with atrial fibrillation. This would have required HRV to near zero after complete autonomic blockade, like HRV in the control group. Instead, a substantial degree of HRV persisted after complete autonomic blockade, which, as pointed out earlier, reflects the "inherent" irregularity of the ventricular rhythm in atrial fibrillation. Hence, HRV would appear to be a relative measure of vagal tone in patients with atrial fibrillation, changes in HRV being significantly related to changes in vagal tone. However, having said that, it should be realized that even in subjects in sinus rhythm, HRV is not an absolute measure of vagal tone, that is, autonomic tone; it is generally recognized that HRV merely reflects fluctuations in autonomic tone

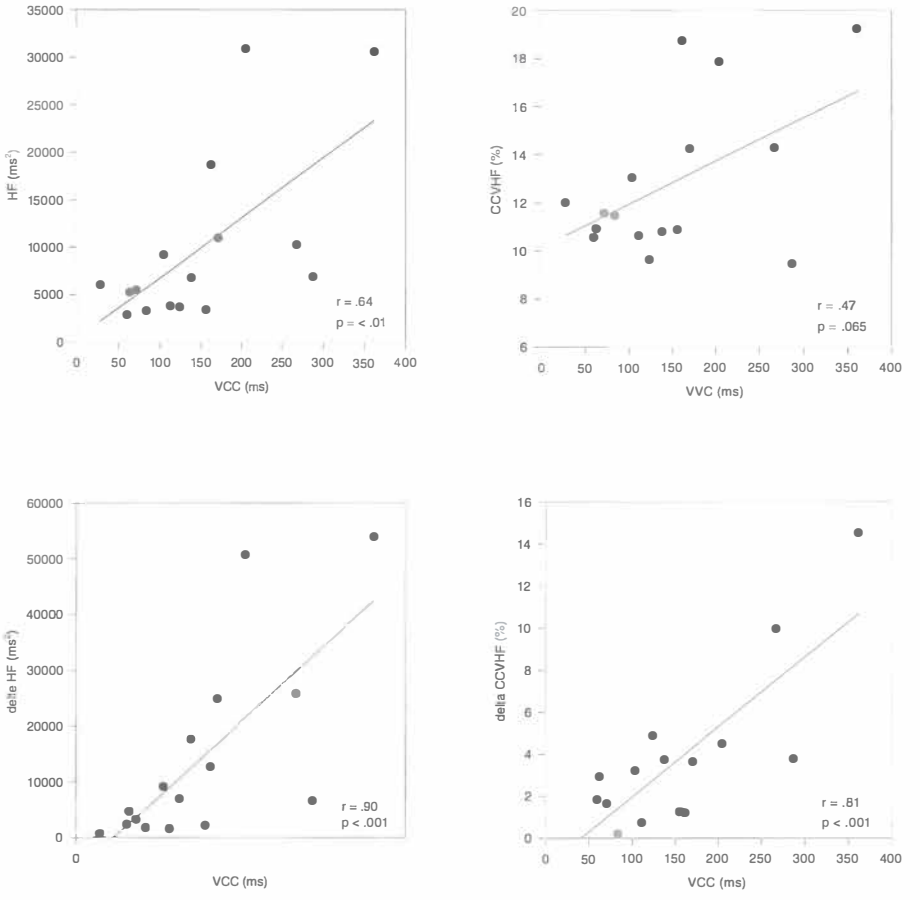


Figure 47. The relation in individual patients between baseline high-frequency power (HF) (upper left panel) and coefficient of component variance of high-frequency power (ccvHF) (upper right panel) and vagal cardiac control (VCC) and the change in high-frequency power (delta HF) (lower left panel) and coefficient of component variance of high-frequency power (delta ccvHF) (lower right panel) and vagal cardiac control after methylatropine.

rather than reflecting the mean level of autonomic tone[2, 146]. Thus, HRV, by its very underlying physiological and mathematical principles, can never provide an absolute measure of autonomic tone, irrespective of the type of cardiac rhythm, i.e. atrial fibrillation or sinus rhythm.

Methodological Considerations

The fact that patients were hospitalized for cardioversion to restore sinus rhythm permitted us to obtain some impression of atrioventricular conduction. In all patients in whom sinus rhythm was restored, the PR-interval was normal (< 0.22 second). Although the presence of a normal PR interval excludes gross

atrioventricular conduction disturbances, particularly in the patients with rheumatic and ischaemic heart disease, the atrioventricular node may have been diseased. In addition, most patients used drugs that affect atrioventricular electrophysiological properties for control of ventricular rate. These drugs included digoxin, also known for its vagomimetic effects[73, 162]. These factors hamper the interpretation of our findings. On the other hand, the results are even more remarkable considering these confounding factors. Correlation coefficients in the control group were somewhat lower than those reported by Hayano et al.[85], who also studied healthy men. In that study, the atropine dose was individualized by carefully titrating the dose against heart rate. Also, these investigators used a metronome to control breathing. Still, our model yielded significant correlations between HRV and VCC in the control group, supporting its validity. More importantly, despite the free breathing, significant correlations were also found the atrial fibrillation group, which in fact adds to the importance of our findings, and also adds to the clinical applicability of our approach. Another methodological issue is also related to respiration: Because respiration as such was not recorded, it cannot be formally ascertained that the observed high-frequency patterning of ventricular rhythm was indeed to some extent related to respiration. Finally, the use of VCC (i.e., mean RR after propranolol minus mean RR after methylatropine) as a measure of vagal tone in the setting of atrial fibrillation may be subject to criticism, because the cited studies[67, 85, 114] referred only to sinus rhythm. Given the complex interplay discussed earlier between atrioventricular input and atrioventricular conduction in atrial fibrillation, the analogy between atrial fibrillation and sinus rhythm with respect to the validity of VCC may not be simply assumed. However, both experimental and clinical data support the analogy. Moe and Abildskov[190] have shown in a dog model of atrial fibrillation that vagal stimulation lowers ventricular rate through a concerted effect on atrioventricular input and concealed conduction in addition to a direct effect on atrioventricular refractoriness, with all three factors acting in the same direction. Importantly, the effect on ventricular rate was stronger when the frequency of stimulation of the vagus nerve was increased. Also, as alluded to earlier, direct vagal stimulation through the carotid sinus nerve in a patient with atrial fibrillation was shown to exert a reproducible lowering effect on ventricular rate[218]. These findings indicate that vagal stimulation lowers ventricular rate in atrial fibrillation in a unidirectional manner, proportional to the strength of stimulation, and hence support the validity of VCC as a measure of vagal tone in atrial fibrillation.

Conclusions and Implications

Using a simple non-invasive model, we were able to show for the first time-that HRV in patients with atrial fibrillation is related to vagal tone. The potentially value of this finding is substantial, considering the value of HRV in patients with sinus rhythm. The potential prognostic value of HRV in atrial

Variable	Unit	Definition
Time-domain		
Mean RR	ms	Mean of all RR-intervals
SD	ms	Standard deviation of all 500 RR-intervals
CV	%	SD/mean RR x 100
rMSSD	ms	Square root of the mean of the squared differences between successive RR-intervals
Frequency domain		
LF	ms^2	Energy in the heart period power spectrum between .04 and .15 Hz
HF	ms^2	Energy in the heart period power spectrum between .15 and .40 Hz
ccvLF	%	Square root of LF/mean RR
ccvHF	%	Square root of HF/mean RR

Table 29. Definitions of time- and frequency-domain variables of Heart Rate Variability.
Note: In the controls normal-to-normal (NN) intervals were used instead of ventricular-to-ventricular (RR) intervals.

n	16
Age (yr.)	56 ± 4
Sex (M/F)	10/6
Median duration of arrhythmia (months)*	15 (2-144)
Underlying heart disease (n)	
Rheumatic heart disease	5
Ischaemic heart disease	2
Hypertensive heart disease	2
"Lone" arrhythmia	5
Congenital heart disease	2
Echocardiographic parameters (mm)	
LVEDD	52 ± 2
LVESD	35 ± 2
LA parasternal view	45 ± 2
LA apical view	70 ± 3
RA apical view	61 ± 2
Medication (n)	
Digoxin	8
Verapamil	5
Digoxin and verapamil	3

*Table 30. Clinical Characteristics.LA indicates left atrium; LVEDD, left ventricular enddiastolic diameter; LVESD, left ventricular endsystolic diameter; and RA, right atrium. *Median with range.*

fibrillation has already been demonstrated[221]. It should, however, be realized that underlying mechanisms may differ since we used rather short recordings whereas Stein et al. used 24-hour recordings, thus accounting for long-term modulating factors. Analysis of HRV in atrial fibrillation might also provide important clinical information. In particular, repeated measurements,

	Controls (n=12)	Patients (n=16)
SD	.59*	.60*
CV	.48	.24
rMSSD	.73†	.61*
LF	.66*	.57*
HF	.70*	.64†
ccvLF	.45	.30
ccvHF	.65*	.47‡

*Table 31. Correlations between Heart Rate Variability variables at baseline and vagal cardiac Control. *P<.05, †P<.01, ‡P=.065*

	Controls (n=12)	Patients (n=16)
SD	.65*	.89‡
CV	.55	.75†
rMSSD	.74†	.87‡
LF	.66*	.72†
HF	.78†	.90‡
ccvLF	.61*	.49
ccvHF	.70*	.81‡

*Table 32. Correlations between changes in Heart Rate Variability variables and vagal cardiac control after administration of methylatropine. *P<.05, †P<.01, ‡P<.001*

	Propranolol		Methylatropine	
	No Digoxin (n=8)	Digoxin (n=8)	No Digoxin (n=8)	Digoxin (n=8)
Mean RR	14 ± 12	24 ± 9	-21 ± 9	-15 ± 10
SD	25 ± 21	35 ± 15	-35 ± 18	-25 ± 15
CV	8 ± 10	9 ± 10	-19 ± 17	-13 ± 12
rMSSD	25 ± 26	42 ± 20	-34 ± 18	-27 ± 13
LF	75 ± 72	109 ± 75	-59 ± 25	-37 ± 28
HF	100 ± 102	145 ± 107	-62 ± 18	-45 ± 22
ccvLF	12 ± 17	14 ± 16	-25 ± 21	-11 ± 17
ccvHF	18 ± 18	23 ± 21	-26 ± 17	-17 ± 15

Table 33. Effects of propranolol and methylatropine on Heart Rate Variability variables in patients with and without digoxin. Values indicate the percentage of change from baseline to propranolol and from propranolol to methylatropine, respectively. Responses in patients with and without digoxin did not differ significantly.

comparing HRV at different times, seems of interest. For instance, such an approach would allow assessment of the effect of drugs with neurohumoral modulating activity[44, 86], though one should be careful not to study drugs with concomitant, direct effects on the atria or the atrioventricular node, since these might affect HRV independent of any autonomic effect.

9.3 COMMENTS

Chapter 9.1 demonstrates that HRV may be used to investigate the role of the autonomic nervous system in the pathogenesis of paroxysmal atrial fibrillation. As such, it confirms earlier preliminary studies [49, 55, 165, 210]. Subsequent studies by other investigators have yielded comparable results in that in a subset of patients paroxysms of atrial fibrillation are preceded by typical changes in HRV indicative of either vagally-mediated or sympathetically-mediated atrial fibrillation [32, 45, 77] though it should be acknowledged that not all groups have come to this conclusion. In particular, findings by Cam and co-workers, based on data from the CRAFT study, are inconsistent [95, 96]. Interestingly, a similar approach using HRV to investigate the role of the autonomic nervous system has been used in patients with (paroxysmal) ventricular tachycardias [104]. Onset of the arrhythmia in this instance was preceded by HRV changes indicative of sympathetic activation in a sizeable portion of patients, which in itself is readily conceivable. Future studies should establish the definitive role of HRV in this field, including its potential to guide therapy.

With respect to the value of HRV in chronic atrial fibrillation, additional studies have recently been published by Hayano and co-workers which complement the study reported in chapter 9.2. [87, 89].These investigators focused on long-term fluctuations in heart rate in atrial fibrillation (hours-days), using non-linear techniques by analysing power-law relations. Unlike short-term fluctuations, these circadian fluctuations in heart rate during atrial fibrillation were shown to resemble those observed in sinus rhythm, suggesting similar underlying regulatory mechanisms [89]. In other words, it may be assumed that the regulatory effect of the autonomic nervous system on long-term heart rate dynamics in sinus rhythm is similarly operative in atrial fibrillation. In an additional study [87], using Lorenz plots, the circadian rhythms in heart rate in heart failure patients with atrial fibrillation were compared with the circadian rhythms in patients with sinus rhythm. Similar patterns were again found in that the circadian rhythms were attenuated in both groups. Interestingly, the investigators were also able to show the presence of circadian rhythms with respect to both AV-nodal refractoriness per se and the degree of concealed conduction within the AV node. As such, this study confirmed our earlier finding that the depressant effect of vagal stimulation on heart rate in atrial fibrillation is due to both direct prolongation of AV-nodal refractoriness and augmentation of concealed conduction [244].

Taken together, the above observations show that analysis of HRV (using either linear or non-linear techniques) may be used to obtain information on autonomic activity in patients with atrial fibrillation, analogous to use of HRV in sinus rhythm. Obviously, it will be necessary to establish normal values

especially given the inherent high short-term variability of heart rate in atrial fibrillation. Nonetheless, the value of HRV is sufficiently established to consider clinical applications. In this connection several possibilities come to mind, such as in patients with sinus rhythm, HRV might be used for risk stratification after myocardial infarction in patients with atrial fibrillation. Also, stratification of risk in the setting of heart failure would be an interesting option, and in fact several investigations on this topic have already been published [69, 221] attesting to the feasibility of the approach. With regard to therapeutic interventions, assessment of the autonomic effect of pharmacologic agents would be possible. For instance, the alleged vagomimetic effect of digoxin might thus be determined as well as the autonomic effect of angiotensin-converting enzyme inhibitors. Finally, HRV may be of value with respect to prediction of arrhythmia outcome. In particular, use of HRV to assess the likelihood of recurrence of atrial fibrillation after cardioversion (chemical or electrical) to sinus rhythm would be clinically relevant. The latter two applications are being investigated in this institute at present and results will be available within the near future.

10.
SAMENVATTING

In een normale (gezonde) situatie wordt het ritme in het hart bepaald door de sinusknoop, een groep van cellen die in staat in om een prikkel af te geven waardoor het hart zich samentrekt. De snelheid waarmee dit gebeurt is de hartfrequentie. Hartfrequentie is niet stabiel, deze verandert al naar gelang de behoefte van het lichaam. Bij inspanning sneller, bij ontspanning langzamer. Deze wisselingen in hartfrequentie worden veroorzaakt door het autonome zenuwstelsel en met name door de nervus sympaticus (zorgt voor versnelling) en nervus vagus, oftewel parasympaticus (zorgt voor vertraging). Deze 2 zenuwen zijn, in een evenwicht, verantwoordelijk voor de variaties in hartfrequentie. Men onderscheidt hierbij variaties met verschillende fases (snelheden). Hoog frequente wisselingen in hartfrequentie - zo rond de 1 maal per 4 seconden (=0.25 Hz)- worden ook wel ademhalings-afhankelijke sinusaritmie genoemd en worden veroorzaakt door de nervus vagus. Tragere wisselingen worden (mede) bepaald door de sympathische zenuw en andere systemen (temperatuursregulatie, hormonen enz.). Bij patiënten met schade aan het hart of het zenuwstelsel is te zien dat de variaties in hartfrequentie afnemen. Hierbij spelen terugkoppelingsmeschanismen via de bloeddruk een belangrijke rol. Afname van variaties blijkt gerelateerd te zijn aan een slechtere prognose van bepaalde patiënten categoricën zoals patiënten na een hartinfarct, of patiënten met diabetes mellitus. De analyse van Hart Ritme Variabiliteit (HRV) is het meten van de wisselingen in de hartfrequentie en kan worden gebruikt om hoog risico patiënten te onderscheiden van patiënten met een minder hoog risico. Dit kan van groot belang zijn bij het bepalen van de behandelingsmethode. Hart Ritme Variabiliteit is een onderzoek dat niet belastend is voor de patiënt (de gegevens kunnen worden gehaald uit een Holter, een niet invasief onderzoek dat vaak sowieso al bij dergelijke patiënten wordt toegepast). Aan het meten van HRV zijn een groot aantal technische aspecten verbonden die het resultaat van de meting kunnen beïnvloeden. Het kiezen van verantwoorde meetmethoden is dan ook een belangrijke zaak. Aangezien de Holteronderzoek (vaak) de basis vormt voor HRV metingen, moet de ECG registratie van goede kwaliteit zijn. Hierbij speelt de aansluitprocedure van het Holter een belangrijke rol, immers voor een gedeelte niet geregistreerd ECG kan in later stadium niet meer worden gecorrigeerd. Een Holteranalyse apparaat deelt QRS complexen in, in groepen van complexen die een grote mate van gelijkenis vertonen. Hierbij is de nauwkeurigheid waarmee dit gebeurt een belangrijke factor die het resultaat van de HRV meting kan beïnvloeden. Een breed QRS-complex kan leiden tot een minder consistente detectie van het QRS en op deze wijze artificiële variaties introduceren. Aangezien het autonome zenuwstelsel vooral de sinusknoop beïnvloedt en in veel minder sterke mate de overige delen van het hart, is het van groot belang om te zorgen dat HRV metingen alleen uitgevoerd worden op sinusritme en dat overige slagen (extrasystolen) van deze vorm van analyse worden uitgesloten. Een goede ECG analyse is dus van groot belang. Automatische detectie van ectopische slagen

leidt tot zeer grote meetfouten. Het is niet mogelijk om wetenschappelijk onderbouwd te komen tot een exact percentage ectopie dat acceptabel is voor HRV analyse. Wel kan proefondervindelijk worden vastgesteld dat indien > 15% van de data bestaat uit "niet sinusritme" een dergelijke ECG niet dient te worden gebruikt voor HRV analyse. HRV kan zowel in het tijdsdomein als in het frequentie domein worden gemeten. Tijdsdomein analyses (zoals de SDNN oftewel de standaarddeviatie van alle sinus- RR-intervallen) zijn eenvoudig uit te voeren en volstaan veelal om een globale indruk van de toestand van de patiënt te verkrijgen. Het meest eenvoudige voorbeeld is de gemiddelde hartfrequentie berekend over 24 uur. Deze waarde blijkt na met myocardinfarct van voorspellende waarde voor de overleving. Een combinatie van de gemiddelde hartslag, de SDNN en één variable die de snellere wisselingen in de hartfrequentie aangeeft(rMSSD) is veelal voldoende om een goede indruk van de HRV van een patiënt te verkrijgen. Frequentie domein analyse, ook wel spectraal analyse genoemd, is meer dan tijdsdomein analyse in staat om de hartfrequentie te ontrafelen in de frequentie wisselingen waaruit deze is opgebouwd. Ook kan met frequentie domein analyse beter dan bij tijdsdomein analyse worden gecorrigeerd voor wisselingen in hartfrequentie. De keuze van het type spectraal onderzoek is ook bepalend voor het resultaat. DFT is te prefereren boven FFT, gezien het feit dat er minder voorbereidende stappen nodig zijn (resampling, windowing). Deze voorbereidende stappen kunnen leiden tot een zeer sterk verlies van spectrale power, waarbij dit verlies afhankelijk is van de hartfrequentie. Aangezien HRV metingen over 24 uur stabiel en dus goed reproduceerbaar zijn is daarmee voldaan aan een belangrijke eis om HRV te kunnen toepassen in de praktijk. Normaalwaarden van HRV zijn afhankelijk van geslacht, leeftijd en gemiddelde hartfrequentie. Bij het vergelijken van onderzoeken dient hier dan ook de nodige aandacht aan te worden geschonken. De registratie-lengte van een Holter is vaak niet exact 24 uur. Een kortere opnameduur leidt tot een verandering in het resultaat van een HRV meting. De gemiddelde hartfrequentie van een Holter wordt al significant beïnvloed als de registratieduur 1 uur korter wordt. Voor al de overige HRV variabelen geldt dat een opname duur van minimaal 20 uur noodzakelijk is. Aangezien ademhaling een zeer belangrijke rol speelt in het tot stand komen van HRV is in veel studies de techniek van metronoom-ademhaling toegepast. Hierbij wordt een bepaalde ademhalingsfrequentie opgelegd aan de patiënt. Deze techniek is uiteraard niet toepasbaar over 24 uur, echter ook blijkt dit in de praktijk geen grote voordelen op te leveren onder gecontroleerde omstandigheden. Het uitvoeren van metronoom-ademhaling wordt in het algemeen ervaren als moeilijk en stress verwekkend, wat daardoor al een contra-indicatie kan zijn voor het uitvoeren ven metronoomademhaling bij bepaalde onderzoeken. HRV-analyse wordt vooral toegepast bij patiënten met sinusritme. Ondanks dat boezemfibrilleren zich op het eerste gezicht niet leent voor een dergelijke analyse is de toepassing van deze techniek hier wel degelijk

interessant. Enerzijds kan HRV worden toegepast bij de bestudering van zowel veroorzakende als in standhoudende mechanismen van HRV. De hoge frequentiecomponent van HRV is tijdens boezemfibrilleren gerelateerd aan de vagale activiteit. Door het sterk stabiele karakter van HRV is deze techniek te gebruiken bij de risico stratificatie van patiëntgroepen, echter studies die prospectief de behandeling van op basis van HRV ingedeelde patiënten evalueren zijn tot op heden niet voorhanden.

11.
DANKWOORD

Heel veel mensen hebben mij geholpen om iets tot stand te brengen, iets dat nu is uitgemond in dit proefschrift. Ik zou graag iedereen heel hartelijk bedanken voor dat wat zij hebben bijgedragen. Bij het opschrijven van een dankwoord ontdek je pas hoeveel mensen je (dank) verschuldigd bent. Ik hoop mijn verplichtingen in te toekomst te kunnen aflossen. Zonder mensen tekort te willen doen wil ik hierbij graag enkelen in het bijzonder noemen:

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Yvonne Larrabee: I want to emphasize that seemingly non-essential hyphens may be of significant value. Thanks for the hyphens and also for the haha's when I replaced too much.

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12.
CURRICULUM VITAE

De schrijver van dit proefschrift is geboren op 7 december 1962 in het Academisch Ziekenhuis te Groningen. Na zijn vroege jaren in Ulrum te hebben doorgebracht werd begonnen aan het Wessel Gansfort College te Groningen. Na het succesvol afleggen van het eindexamen HAVO in 1980, werd de opleiding HBO-A medische microbiologie aan de laboratoriumschool gevolgd. In 1983 werd dit eindexamen met goed gevolg afgelegd en een interne opleiding gestart tot Holteranalist bij st. Fysiologic te Zeist. In 1986 werd een overstap gemaakt naar het Academisch Ziekenhuis Groningen alwaar werd aangevangen met een studie informatica. Deze studie werd afgerond in 1989 met het praktijk diploma Fortran. Tot op dit moment is de schrijver werkzaam als biotechnicus aan het Academisch Ziekenhuis te Groningen.

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